

# CYTOLOGIA

International Journal of Cytology  
Internationale Zeitschrift für Zytologie  
Archives Internationales de Cytologie

Edita

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**K. Fujii**

Botanicae Prof. Hon.  
Imperialis Universitatis Tokyensis

WITH THE COOPERATION OF MANY SPECIALISTS AND THE SUPPORT  
OF THE "WADA-KUNKÔKAI" FOUNDATION

UNTER MITWIRKUNG ZAHLREICHER FACHGELEHRTER, MIT UNTERSTÜT-  
ZUNG DER „WADA-KUNKÔKAI“ STIFTUNG

AVEC LE CONCOURS DE NOMBREUX SPÉCIALISTES, PAR LES SOINS  
DE LA FONDATION «WADA-KUNKÔKAI»

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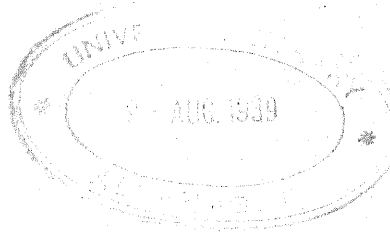
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## The Living Egg-Cell of *Triturus torosus*

By

J. Frank Daniel

University of California

As soon as the egg of *Triturus torosus* passes from the cloaca into the water its jelly coats swell, displaying two concentric zones: a transparent, irregular outer zone consisting of a single layer, and a cloudy, spherical inner zone. The inner zone is composed of three layers, a semisolid inner layer, a surrounding wrinkled stratum, and a tough, striated layer ( $J^3$ , fig. 1), bounding the inner zone and separating it from the outer clear layer.

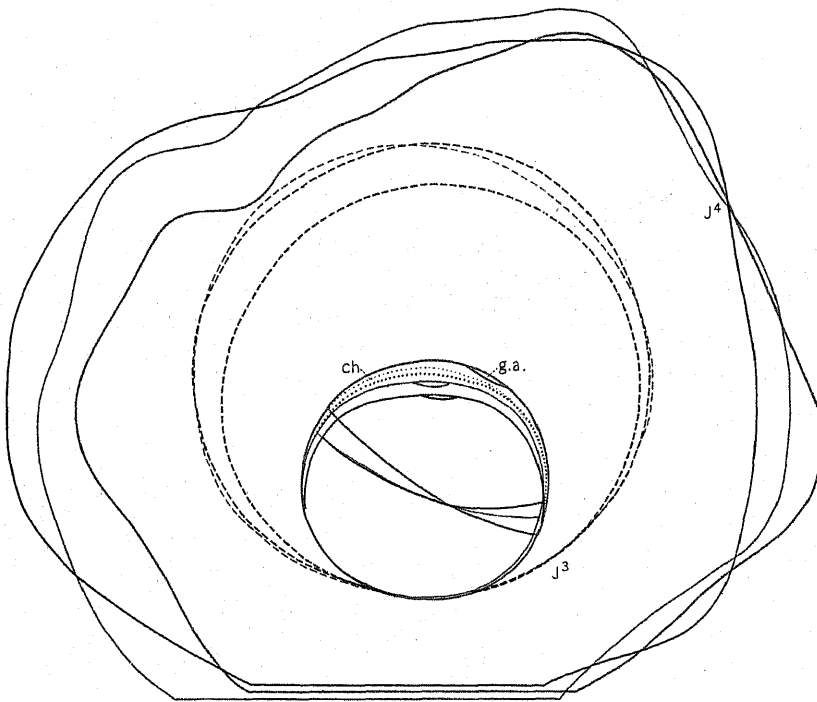


Fig. 1. Zygote, *Triturus torosus*. (Series 13). Red 9:45; blue 10:45; black 11:45.  
ch., chorion; g.a., germinal area;  $J^3$ -4, third and fourth jelly layers.

When first laid the egg lies at or near the center of the inner sphere of jelly. Soon, however, the swollen inner layer begins to clear and to liquefy. Clearing is first apparent at the top and near the periphery of the inner layer. If at this stage a pencil of light is thrown on the face of the prism from behind the microscope (see



paragraph below), myriads of tiny, slowly moving flecks are visible in the clearing jelly. Some of these settle down on the egg, giving it a fuzzy appearance; others cover the floor, forming what I have designated the *white plac.* As the inner jelly becomes more liquid the egg responds to gravity by settling and rotating so that the germinal area comes to lie upward and the heavy yolk pole downward. After the egg has reached the bottom the animal pole flattens somewhat, forming the superior plateau over which is seen the chorion (*ch.*, fig. 1).

We may now follow through the changes which take place in an egg and in its surrounding jelly layers. To do this an essential

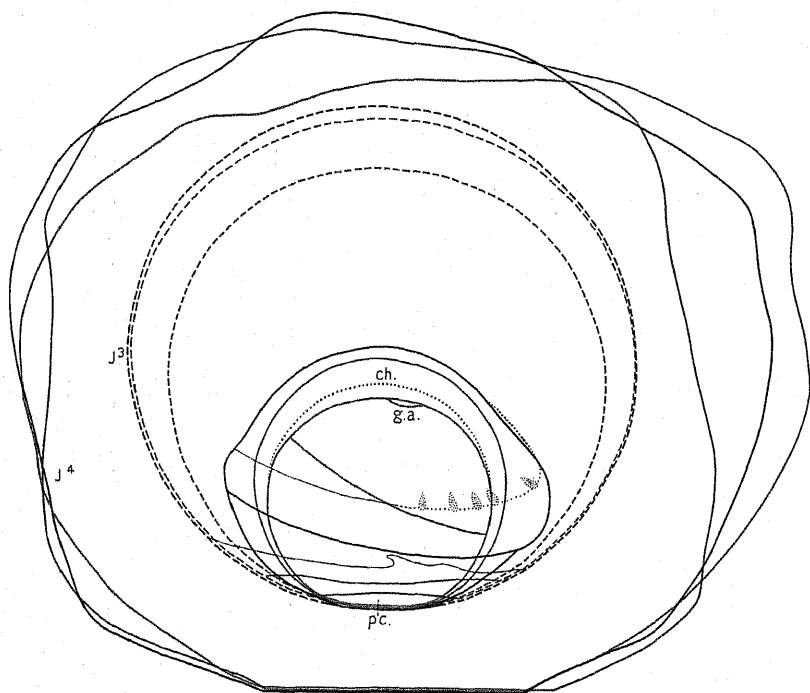


Fig. 2. Zygote, gastrula, neurula (concentric). (Series 13). Red 11:45, March 21; blue 8:50, March 23; black 8:45, March 26. *ch.*, chorion; *g.a.*, germinal area; *J*<sup>3-4</sup>, third and fourth jelly layers; *pc.*, white plac.

is observation in profile. This is rendered possible through the use of the Rotary Disc (Daniel and Burch, 1933). Record of the changes which take place in the egg may be secured by *camera lucida* drawings made at frequent intervals (every fifteen minutes) during the first three hours of development, and less regularly for the ensuing days. In this first study nineteen series were completed, some of which were followed closely for ten days. We may illustrate this study by series 13.

In figure 1, series 13, the zygote, in solid red line, was first sketched at 9:45 A.M., soon after it had been taken from the cloaca of the female. At that time it had just settled and had almost completely rotated so that the germinal area (*g.a.*) approached the top. The combined inner zone of jelly had increased to two-thirds the thickness of the outer layer. At the end of an hour the zygote, sketched in solid blue line, had decreased considerably in size; it had settled to the floor of the inner cavity; had fully rotated and flattened so that the corionic arch (*ch.*, fig. 1) stood well above the egg. Both the outer layer, in solid blue line, and the inner space bounded by the wrinkled striate layer were greatly enlarged. An hour later the features of the egg, in solid black line, were similar to those at 10:45, excepting that the egg had decreased still further in size. Within its normal jelly, however, it is to be noted that the egg flattens less than have experimental eggs formerly studied (Daniel, 1932, p. 307).

Two later stages of this egg are added in figure 2. The sketch, in solid red line, is essentially like the last stage considered. The first of the later stages, at three days of age, sketched in solid blue line, was of the gastrula, which had so increased in volume as to fill the chorion. The white plac (*pc*, fig. 2) formed on the floor of the inner jelly cavity during the process of liquefaction, was a marked feature of this stage.

At five days the egg in its surrounding jelly envelopes is shown in solid black. The neural tube had closed; the neurula had increased in size and had assumed beginning larval form. At the end of a week the larva extended across the inner cavity.

In this series we have studied the activities of the egg in its natural surroundings, observing the changes as they took place. We have seen the thin inner and outer zones of jelly expand to enormous proportions. We have observed phenomena associated with the liquefaction of the inner layer, and the responding egg settle to the bottom and rotate. We have followed the elevation of the chorion, the flattening and diminution in size of the zygote, the increase in mass of the blastula and gastrula, and finally the assumption of specific larval form.

#### References

- Daniel, J. Frank, 1932. Morphogenesis. Univ. Calif. Publ. Zool., 36: 299-324.  
— and A. B. Burch, 1933. A rotary disc for the observation of objects in profile. Univ. Calif. Publ. Zool., 39: 201-204.

## Zur Kenntnis der Plastiden- und Plasmavererbung

Von

O. Renner, Jena

### 1. Scheckung bei Artbastarden von *Eu-Oenothera*

Das Problem der plasmatischen Vererbung scheint durch die Untersuchungen von SCHWEMMLE (1935) über Kreuzungen komplex-heterozygotischer Arten von *Eu-Oenothera* einen neuen Aspekt erhalten zu haben. SCHWEMMLE findet bei solchen Bastarden, die ihre 14 Chromosomen in der Diakinese der Reduktionsteilung in eine einzige Kette anordnen und keine Spaltung zeigen, im Laufe einiger Generationen deutliche Veränderungen der Laubfarbe, der Wüchsigkeit, der Blattgröße, der Länge der Blütenröhre, und glaubt annehmen zu dürfen, daß diese Veränderungen auf wechselseitiger Abänderung der Plasmen und der Genome beruhen.

Herr Kollege SCHWEMMLE hatte die Freundlichkeit, mir Samen von *Oenothera Berteriana* (Komplexe B und l)<sup>1)</sup> und *odorata* (Komplexe v und J) sowie ihrer Bastarde in der ersten und in späteren Generationen zu überlassen. So konnte ich im vergangenen Sommer die interessanten Formen selber beobachten. Wenn *Oe. Berteriana* Mutter und *Oe. odorata* Vater ist, entstehen kräftige, satt grüne Drillingsbastarde: B.J mit stark welligen Blättern (32 Pflanzen), l.v mit grünen Stengeln (11 Pflanzen), l.J mit roten Stengeln und rotrandigen Blättern (17 Pflanzen); die vierte zu erwartende Verbindung B.v fehlt. In der reziproken Kreuzung ist J.B viel schwächer, blaßgrün, kann aber doch noch verhältnismäßig leicht zur Blüte erzogen werden (17 Pflanzen); v.l ist weißlichgrün und so schwach, daß ich kein Exemplar auspflanzen konnte, während SCHWEMMLE die Form bis zur Blüte brachte; die dritte zu erwartende und wohl grüne Form, v.B, bekam ich nicht zu Gesicht; J.l fehlt nach SCHWEMMLE immer.

An der F<sub>1</sub> von *Oe. Berteriana* × *odorata* fiel mir etwas auf, wovon SCHWEMMLE nicht spricht: die Kombinationen l.v und l.J hatten teilweise gelbscheckiges Laub. An dem einzigen gescheckten l.J-Individuum waren die meisten Zweige bis zu den Brakteen periklinal gelb-über-grün, bei den 3 gescheckten l.v-Pflanzen wurde die Variegation

1) Die Buchstabensymbole, die SCHWEMMLE für die Komplexe wählt, sind schwer zu merken, und auch deswegen nicht sehr zweckmäßig, weil wir mit Buchstaben einzelne Faktoren anzudeuten gewohnt sind.

gegen die Blütenregion undeutlich. Bei B.J fand ich keine Scheckung, ebensowenig bei der reziproken J.B; die früh sterbenden Sämlinge von v.l habe ich nicht sorgfältig auf Scheckung untersucht. Es kommt also, in Verbindung mit verschiedener Laubfärbung der reziproken Kreuzungen, wie bei *Oenothera-Onagra* Bastardscheckung vor, die auf Plastidenübertragung durch den Pollen zurückzuführen ist. Die Genomkombination l.v z.B. läßt die Plastiden der *Oe. Berteriana* voll ergrünen, aber nicht die der *Oe. odorata*; auch bei den Kombinationen l.J und B.J sind die *Berteriana*-Plastiden tauglicher als die empfindlicheren *odorata*-Plastiden.

Das Auftreten von Scheckung zeigt, daß an der Verschiedenheit der Laubfärbung der reziproken Bastarde die spezifische Konstitution der Plastiden einen wesentlichen Anteil hat. Und die von SCHWEMMLE beobachtete „Erholung“ der zunächst blassen Bastarde mit *odorata*-Plasma ist vielleicht so zu verstehen, daß es sich hier um die Nachkommen solcher Individuen handelt, die vom Vater *Berteriana* verhältnismäßig viele Plastiden bekommen haben. Die (*od.*  $\times$  *Bert.*) l.v  $F_6$  (4 Pflanzen) ist genau so schön grün und kräftig wie (*Bert.*  $\times$  *od.*) v.l  $F_1$ . Die (*od.*  $\times$  *Bert.*) J.B  $F_9$  dagegen (5 Pflanzen) ist zwar kräftiger als die  $F_1$  derselben Verbindung, aber doch noch lange nicht so kräftig wie (*Bert.*  $\times$  *od.*) B.J  $F_1$ ; hier würde, wenn meine Deutung richtig ist, das Cytoplasma neben den Plastiden noch eine deutlich differenzierende Wirkung auf die Laubfarbe ausüben, anders als ich es bis jetzt von *Onagra* kenne.

Wenn die „Erholung“ einer blassen Bastardform auf die Ausortierung der besser ergrünenden väterlichen Plastiden zurückgeht, müßte man schon in der  $F_1$  gut grüne Zweige finden, und nur solche dürften „erholte“ Nachkommenschaft erzeugen; auf blassen Zweigen selbstbestäubte Blüten dürften wieder nur blasser Sämlinge bringen. Aber vielleicht sind solche blassen Zweige steril. Daß die blasser l.v oft schlechte Antheren hat, gibt SCHWEMMLE an (S. 183), und ich habe an dem gescheckten Individuum von l.J in den Blüten, deren Tragblätter gelbrandig waren, fast keinen Pollen gefunden, während die Antheren auf ganz grünen Zweigen pollenreich sind. Wenn meine Vermutung richtig ist, müßte die „Erholung“ in  $F_1$  auch schon abgeschlossen sein. Solange diese Fragen nicht beantwortet sind, bleibt alles hypothetisch.

Auch die von SCHWEMMLE gefundene Abänderung der Genome in späteren Generationen kann vielleicht auf ein geläufiges Schema gebracht werden. Wenn die in den fraglichen Bastarden vereinigten Komplexe auch keine ganzen Chromosomen austauschen, so könnten sie doch Chromosomenstücke auswechseln; in den Chromosomenringen von *Onagra* ist ja crossing over vielfach angetroffen.

Falls dann unter den cross-overs günstiger konstituierte Typen sind als die ursprünglichen, können die neuen Typen leicht unwillkürlich ausgelesen werden. Auch die Erholung inbezug auf die Laubfarbe könnte mit solcher Selektion gewisser Genotypen zusammenhängen; wie sehr die Laubfarbe von der genischen Beschaffenheit der Komplexe abhängt, habe ich bei *Onagra* oft beobachten können (ebenso mein Schüler KRUMBHOLZ), wenn es sich hier auch um Austausch ganzer Chromosomen zu handeln pflegt. Einen Zusammenhang zwischen Faktorenaustausch und Plastiden hat SCHWEMMLE's Schüler OELKRUG auch für *Eu-Oenothera* schon angenommen, der in der späteren Nachkommenschaft der Kreuzung *Oe. Berteriana*  $\times$  *mollissima*, nicht schon in der  $F_1$ , Schrecken angetroffen hat.

SCHWEMMLE nimmt, wenn ich ihn richtig verstehe, an, daß in einer gegebenen Bastardsippe die „Umstimmung“ des Plasma und die Veränderungen der Genome sich gleichermaßen in allen Individuen abspielen. Aber bei der Schwächlichkeit der betreffenden Bastarde kann ganz wohl Selektion durch differenzierte Sterblichkeit vorliegen. Auch die noch ausstehende Entscheidung, ob die genotypischen Abänderungen reversibel oder irreversibel sind (S. 185), würde über das Wesen dieser Veränderung noch nicht viel aussagen. Denn wenn Selektionsvorgänge dabei eine Rolle spielen, werden sie bei Rückkreuzung wohl auch in rückläufiger Richtung zu treiben sein.

Für eine sichere Ergänzung zu den Befunden SCHWEMMLE'S halte ich nur die Feststellung, daß die verwendeten Arten von *Eu-Oenothera* spezifisch konstituierte Plastiden besitzen und durch den Pollen übertragen, was zu den von *Onagra* bekannten genetischen Folgen führt. Daß Aussortierung von Plastiden bei der „Erholung“ der blassen Bastarde wirksam ist, und daß Selektion neuer genischer Kombinationen die beobachtete Veränderung der Komplexe herbeiführt, möchte ich nur als zu prüfende Möglichkeit, neben den von SCHWEMMLE ins Auge gefaßten Momenten, zur Diskussion stellen. Wenn SCHWEMMLE das Richtige trifft, haben wir einen sehr merkwürdigen Fall von Anpassung vor uns. Das Plasma—mit Einschluß der Plastiden—würde sich in fremder karyotischer Umgebung in adaptativem Sinn verändern, und vielleicht auch umgekehrt die Genome in fremder plasmatischer Umgebung; zur Ergänzung wäre das Studium der gut grünen *Berteriana*  $\times$  *odorata*-Bastarde, bei denen die Lebensfähigkeit nicht von solchen Umstimmungsvorgängen abhängt, in späteren Generationen erwünscht. Die Entscheidung, ob derartige wechselweise Anpassung der genetischen Konstitutionselemente vorkommt, ist sicher von größter Wichtigkeit.

## 2. F<sub>2</sub>-Generationen blasser *Onagra*-Bastarde

Nach den Mitteilungen SCHWEMMLE's erscheint es nötig, blasse Bastarde von *Onagra* in einer größeren Zahl von Generationen aufzuziehen als ich bisher getan habe. Über F<sub>3</sub> hinaus habe ich solche Bastarde früher nicht verfolgt, und ich kann auch jetzt über weiter fortgesetzte Züchtungen noch nicht berichten. Aber eine Versuchsreihe ist in Angriff genommen.

In der zweiten Generation der gelbgrünen *Oenothera* (*biennis* × *suaveolens*) *rubiflava* und reziprok habe ich auch diesmal wieder nicht das geringste Anzeichen eines Kräftigerwerdens gegenüber der F<sub>1</sub> finden können; mit der F<sub>1</sub> zu vergleichen ist natürlich nur die Verbindung *rubens* · *flavens*, nicht die in großer Zahl herausspaltende *lutescens* = Sp-*flavens* · sp-*flavens* bzw. sp-*flavens* · sp-*flavens*, die aber ebenso blaß ist wie die *rubiflava*.—Was den früher (1936, S. 243) erwähnten Unterschied zwischen den beiden reziproken *rubiflavae* betrifft, so ist die *rubiflava* aus *suaveolens* als Mutter auch in der 2. Generation deutlich schwächer gefunden worden als die reziproke, wie in der F<sub>1</sub>.

Auch die aus *Lamarckiana* × *Hookeri-curva* weißrandig gewonnene blaßgrüne *Hookeri-velutina* (1936, S. 271) lieferte bei Selbstbestäubung nur blasse Sämlinge, wohl teils <sup>h</sup>*Hookeri* · <sup>h</sup>*Hookeri* teils <sup>h</sup>*Hookeri* · *velans*, von denen nur wenige so kräftig wurden, daß sie ausgepflanzt werden konnten.

Die *rubipicta* = *pingens* · *rubens* aus *Oe. cruciata* × *biennis* ist an und für sich dunkelgrün, wird aber durch eingepflichte *biennis*-Plastiden oft sehr stark gelbscheckig, einige Individuen werden sogar ganz gelb und gehen ein ohne zu blühen. Ob eine zweite Generation mit *biennis*-Plastiden lebensfähig ist, läßt sich also nur in der Weise prüfen, daß gelbe oder wenigstens gelbmantelige Zweige gescheckter Individuen selbstbestäubt werden. Sämlinge von solchen Zweigen sind immer gelb und sterben, ohne nach der Entfaltung der Kotyledonen Primärblätter zu erzeugen. Daß die blassen Plastiden im gelben Mantel periklinal-bunter *rubipicta* gesunde *biennis*-Plastiden sind, zeigen die Verbindungen gelbrandiger *rubipicta* mit (*biennis* × *Lamarckiana*) *albivelutina* ♂ und mit *Lamarckiana* ♀. In beiden Fällen ist die Nachkommenschaft—*pingens* · *velans* und *rubens* · *velans*, bzw. *velans* · *rubens*—rein grün, ohne Spuren von Scheckung.

Ebenso lieferte ein ganz gelbgrüner Ast einer gescheckten *rubieflexa* nur gelblichweiße bis hellgrüne, sehr bald sterbende Sämlinge. Die *rubieflexa* war aus *rubicurva* × *rubieflexa* weiß gewonnen (1936, S. 273), ihre grünen Plastiden stammen aus *muricata*, ihre blassen aus *biennis*.

Es soll nun das Verhalten der *biennis*-Plastiden in der *rubipicta* auf die Weise über mehrere Generationen verfolgt werden, daß immer rein grüne Individuen mit gelbmanteligen Pflanzen derselben Familie gekreuzt werden. Falls eine „Umstimmung“ der Plastiden im Sinne einer Anpassung an die Wirkung des *pingens*-Komplexes eintritt, müßten die blassen Bezirke der Schecken mit der Zeit besser grün werden, im günstigen Fall so weit, daß aus den blassen Anteilen durch Selbstbestäubung lebensfähige Nachkommenschaft zu gewinnen wäre. Aber auch wenn dieses Ziel erreicht würde, was mir nach meinen bisherigen Erfahrungen ganz unwahrscheinlich ist, wäre noch zu prüfen, ob die Veränderung wirklich auf Umstimmung der Plastiden und nicht auf Selektion neuer genischer Kombinationen beruht.

#### Belege

- Nr. 1. (*Lamarckiana* × *Hookeri-curva* weißrandig) *Hookeri-velutina* (aus 1935/210) selbstbestäubt: 75 blaßgrüne Sämlinge, nur 12 konnten ausgepflanzt werden. 1936/196.
- Nr. 2. (*R-biennis* × *suaveolens*) *R-rubiflava* 1 (aus 1935/248) selbstb.: 50 gelbgrüne Sämlinge, 32 ausgepflanzt, der Rest vorher gestorben; blühend 5 gelbgrüne schwache *R-rubiflava* und 25 ebensolche *r-lutescens*. 1936/198.
- Nr. 3. (*R-biennis* × *suaveolens*) *R-rubiflava* 2 (aus 1935/248) selbstb.: 50 Sämlinge wie vorher, blühend 3 gelbgrüne schwache *R-rubiflava* und 28 *r-lutescens*. 1936/199.
- Nr. 4. (*suaveolens* × *R-biennis*) *R-rubiflava* (aus 1935/250) selbstbest.: 80 sehr blasse und schwache Sämlinge, schwächer als 198 und 199, meist sterbend; 9 bleiben am Leben und werden ausgepflanzt, davon 2 *R-rubiflava* und 7 *r-lutescens*, beide Formen gelbgrün und schwach. 1936/200.
- Nr. 5. (*cruciata* × *R-biennis*) *rubipicta* 2 (aus 1935/249), gelbrandiger Ast selbstb.: 49 gelbgrüne Sämlinge, alle gestorben. 1936/201.
- Nr. 6. (*cruciata* × *R-biennis*) *rubipicta* 1 (aus 1935/249), gelbrandiger Ast × (*biennis* × *Lamarck.*) *albivelutina* F<sub>n</sub>: 49 grüne Sämlinge, 1 gelblichweißer gestorben; blühend 41 *pingens-velans*, 7 *rubens-velans*, alle grün, kräftig, nicht gescheckt. 1936/202.
- Nr. 7. *r-Lamarckiana* × *rubipicta* gelbrandig (aus 1935/249): 80 *velans-rubens*, alle grün, kräftig, nicht gescheckt. 1936/215.
- Nr. 8. (*rubicurva* × *rubiflexa* weißrandig) *R-rubiflexa* (aus 1935/239), blaßgrüner Ast selbstbest.: 85 gelblichweiße bis hellgrüne Sämlinge, alle gestorben. 1936/203.

### 3. Erhaltung der spezifischen Qualität der Plastiden

Früher (1936, S. 275) ist über eine grüne *rubipicta* berichtet, die aus *cruciata* weißrandig × *biennis* entstanden war; der weiße Rand bzw. Mantel dieser *cruciata* enthielt *Lamarckiana*-Plastiden in *cruciata*-Plasma. Es war etwas überraschend, daß die Verbindung *pingens-rubens* mit *Lamarckiana*-Plastiden ebensogut ergrünt wie mit *cruciata*-Plastiden, weil sie mit *biennis*-Plastiden blaß ist und die Chromatophoren von *biennis* und *Lamarckiana* sich recht ähnlich verhalten. Die weitere Analyse hat aber die frühere Deutung bestätigt.

Es wurden von (*cruc.* weißr.  $\times$  *biennis*) *rubipicta* zunächst zwei ganz grüne Individuen verwendet, ohne Spuren der so häufigen Variegation, die durch die *biennis*-Plastiden verursacht wird. Bei Selbstbestäubung entstehen nur grüne Pflanzen. Bei Bestäubung mit dem Pollen von *cruciata* entstehen aber zur Hauptsache weiße Sämlinge, was beweist, daß die grünen Plastiden der als Mütter verwendeten *rubipictae* eben nicht *cruciata*-, sondern fremde Plastiden waren, und zwar nach der Entstehungsgeschichte *Lamarckiana*-Plastiden. Daß auch ziemlich viel grüne *pingens*  $\cdot$  *flectens* = *cruciata* entsteht, stimmt mit früheren Erfahrungen (1936, S. 275) gut überein; die durch den Pollen eingeführten *cruciata*-Plastiden vermehren sich gut, weil sie mit allen zugehörigen idiotypischen Elementen zusammenkommen. Neben *cruciata* wären auch einige *rubiflexa*-Individuen zu erwarten, weil die *rubipicta* nicht nur *pingens*-, sondern auch *rubens*-Eizellen hat; vielleicht sind die *rubiflexa*-Sämlinge alle bleich geblieben.

Aus derselben *rubipicta*-Familie wurden gelbrandige Zweige zweier gescheckten Individuen verwendet, mit *biennis*-Plastiden im blassen Mantel. Selbstbestäubung gibt nur blasse, bald sterbende Sämlinge, wie bei der gewöhnlichen (*cruciata*  $\times$  *biennis*) *rubipicta*. Die Bestäubung eines solchen gelbrandigen Zweiges mit dem Pollen von *cruciata* gibt wieder im wesentlichen bald eingehende, ganz blasse Sämlinge, einige gescheckte oder ganz grüne bleiben am Leben, sind *cruciata*.

Auch die *pictivelutina*, die aus *cruciata* weißrandig  $\times$  *albivelutina* gewonnen war (1936, S. 276), ist daraufhin geprüft worden, ob sie wirklich zur Hauptsache *Lamarckiana*-Plastiden besitzt, wie früher angenommen; durch den Pollen eingeführte *biennis*-Plastiden werden vorkommen, ohne sich in der Färbung zu unterscheiden. Bei Selbstbestäubung entstanden nur grüne Pp- und PP-*pictivelutina*. Bei Bestäubung mit dem Pollen der *cruciata* dagegen traten wie erwartet war weiße, grün-weiß-gescheckte und gelbgrüne Sämlinge auf. Was von den Schecken am Leben blieb, erwies sich als *cruciata* = *pingens*  $\cdot$  *flectens*, teils normal teils P-*cruciata* (weil P von *velans* auf *pingens* übergeht). Die gelbgrünen Sämlinge waren *velans*  $\cdot$  *flectens*, von derselben Farbe wie primäre (*Lamarckiana*  $\times$  *cruciata*) *flexivelutina*. Die Plastiden der grünen *pictivelutina* haben sich also als typische *Lamarckiana*-Plastiden ausgewiesen.

Bemerkenswert ist, daß die gelbgrüne *flexivelutina* dieser Rückkreuzung von *pictivelutina* mit *cruciata*, ebenso wie der schon oft aufgezugene primäre aus *Lamarckiana*  $\times$  *cruciata* gewonnene Bastard, keine Variegation mit dunkelgrün zeigt, während die weißen *cruciata*-Sämlinge die aus dem Pollen übergetretenen *cruciata*-Plastiden in



ihren satt grünen Bezirken so deutlich zur Schau tragen. Aus gewöhnlicher (*cruciata* × *Lamarckiana*) *pictivelutina* durch Rückkreuzung mit *cruciata* ♂ gewonnen, also mit Plasma und Plastiden von *cruciata* ausgestattet, ist die *flexivelutina* dunkelgrün. Die Erscheinung ist wohl nur durch die Annahme zu erklären, daß die durch den Pollen eingeführten *cruciata*-Plastiden sich in Anwesenheit des *velans*-Komplexes und der *Lamarckiana*-Plastiden schlechter vermehren als diese. Diesen Vorteil haben die Plastiden der *Lamarckiana* vor denen der *cruciata* in der *flexivelutina* selbst dann, wenn das Cytoplasma von *cruciata* stammt; das ist wohl das interessanteste Ergebnis dieser Kreuzung.

#### Belege

- Nr. 9. *rubipicta* grün 1 (aus *cruciata* weißrandig × *biennis*, 1935/217) selbstb.: 49 grüne *rubipicta*. 1936/207.
- Nr. 10. *rubipicta* grün 3 selbstb.: 56 grüne *rubipicta*. 1936/213.
- Nr. 11. *rubipicta* grün 1 × *cruciata*: 24 weiß, weiß-grün-gescheckt, 17 grün; ausgepflanzt 22 *cruciata*, davon 17 grün, 5 gescheckt. 1936/208.
- Nr. 12. *rubipicta* grün 3 × *cruciata*: 12 weiß, 8 grüne *cruciata*, davon 3 weißscheckig, im Beet nur noch 1 stark gescheckt. 1936/214.
- Nr. 13. *rubipicta* gelbrandig 2 selbstb.: 33 gelbgrüne Sämlinge, alle gestorben. 1936/211.
- Nr. 14. *rubipicta* gelbrandig 4 selbstb.: 35+14 wie vorher. 1936/209.
- Nr. 15. *rubipicta* gelbrandig 2 × *cruciata*: 28 weiße Sämlinge, davon 11 grünscheckig; ausgepflanzt nur noch 5, *cruciata*, davon 3 ganz grün, 2 gescheckt. 1936/212.
- Nr. 16. *pictivelutina* (aus *cruciata* weißrandig × *albivelutina*, 1935/218) selbstb.: 45 grüne *pictivelutina*, teils pP teils PP. 1936/2 6.
- Nr. 17. *pictivelutina* × *cruciata*: 30 weiße Sämlinge gestorben, 15 grünweiß-gescheckte, 13 gelbgrüne; ausgepflanzt 26; blühend 12 gelbgrüne *flexivelutina*, 10 grüne *cruciata*, teils p teils P, 7 davon weißscheckig, auch P dabei. 1936/29.

#### 4. Verhalten defekter Plastiden bei Paralbomaculatio

Von durch Mutation defekt gewordenen Plastiden habe ich die der albomaculaten *M-albiflava* weiter verfolgt, die 1936, S. 282 beschrieben ist, und zwar in der Verbindung *pingens* · *M-flavens* aus der Kreuzung *cruciata* × *M-albiflava* weißrandig. Diese *M-pictiflava* war teilweise stark weißscheckig, und sowohl weißmantelige wie ganz weiße Zweige wurden für die Weiterzucht verwendet. Die Selbstbestäubung ganz weißer Zweige gab nur farblose Sämlinge—zuerst sind die Kotyledonen blaß grünlichgelb, dann werden sie fast weiß—, die von weißrandigen Zweigen lieferte neben weißen teilweise auch grün-weiß-gescheckte bis ganz grüne Pflanzen, ein Beweis dafür daß die Keimzellen nicht immer aus dem blassen Mantel, sondern mitunter aus dem grünen Kern hervorgehen, wie es auch bei weißrandiger *cruciata* beobachtet ist (1936, S. 275). Damit ist also bei allen Verbindungen zu rechnen. Ob auch „gemischte“ Keimzellen mit grünen und farblosen Plastiden vorkommen, läßt sich noch nicht entscheiden.

Bei Bestäubung weißer bzw. albotunicater Zweige der *M. pictiflava* mit dem Pollen rein grüner Pflanzen derselben Familie mußten lebensfähige Grünschecken entstehen, ebenso wenn Pollen von *Oe. cruciata*, *muricata*, *rubiflava* mit *muricata*-Plastiden verwendet wurde. Das ist auch immer der Fall gewesen, und zahlreiche Individuen dieser Verbindungen sind sogar ganz grün geworden. Solche ganz grünen Pflanzen können allerdings auch aus grünen Eizellen der albotunicaten Zweige hervorgegangen sein, aber nicht selten brachten zunächst ungescheckte Individuen zur Blütezeit noch farblose Grundseitsprosse hervor, waren also doch aus Zygoten mit farblosen und grünen Plastiden entstanden. Besonders gut grün wurde in der Verbindung *pictiflava*  $\times$  *cruciata* die rekonstruierte *cruciata*, aus der oben (vgl. S. 649) angedeuteten Ursache. Die kleine Tabelle illustriert das Verhalten der *cruciata* gegenüber dem der *flaviflexa*.

Von der *cruciata* war also fast die Hälfte ganz grün geworden, von der *flaviflexa* noch nicht einmal 10%. Leider lassen die ganz blassen Sämlinge sich noch nicht bestimmen, es ist also unbekannt, wie hier das Verhältnis zwischen *cruciata* und *flaviflexa* sich stellt.

Von besonderem Interesse waren die Verbindungen der weißen *pictiflava* mit *biennis* ♂ und *suaveolens* ♂. Die primären Kreuzungen *cruciata*  $\times$  *biennis* und *cruciata*  $\times$  *suaveolens* geben gescheckte *rubipicta* und *pictiflava*, wobei die durch Pollenplastiden blassen Anteile des Laubs aber beträchtlich besser gefärbt sind als die zuletzt ganz weißen Defektbezirke der albomaculaten *pictiflava*. Die Sämlinge der neu geprüften Verbindungen lassen nun, wie zu erwarten, die ganz blassen, mit kranken Plastiden ausgestatteten und die blaßgrünen, mit gesunden, aber nicht voll ergrünungsfähigen Plastiden beimpften Areale deutlich unterscheiden, bevor sie zugrunde gehen.

Die Kreuzung *pictiflava* weißrandig  $\times$  (*biennis*  $\times$  *Lamarck*.) *albivelutina* F<sub>n</sub> sollte zeigen, ob die Verbindung *pingens*  $\cdot$  *velans* mit *biennis*-Plastiden voll ergrünen und leben kann. Eine frühere Beobachtung an *cruciata* weißrandig (mit blassen Plastiden von *Lamarckiana*)  $\times$  *albivelutina* hat die sichere Entscheidung wünschenswert gemacht (1936, S. 276), und die Ausheilung einer an und für sich blassen Verbindung durch Einführung geeigneter Pollenplastiden ist sicher eindrucksvoller als das Ausbleiben der Variegation bei einer an und für sich grünen Verbindung. Tatsächlich wurden einige Schecken der genannten Kreuzung zu blühbarer, albomaculater *pictivelutina*, deren grüne

Nr.	<i>cruciata</i> grün	<i>cruciata</i> bunt	<i>flava</i> grün	<i>flava</i> bunt
221	0	1	2	7
230	7	7	0	15
227	4	3	0	13
228	4	6	2	6
Summe	15	17	4	41

Plastiden nun von *biennis* stammen; andere werden zu ebensolcher *flavivelutina*. Bemerkenswert ist hier wieder, wie wenige Sämlinge eine genügend große Zahl ergrünungsfähiger Pollenplastiden besaßen, um am Leben zu bleiben. Augenscheinlich vermehren sich die *biennis*-Plastiden im Zusammenleben mit dem *cruciata*-Plasma und mit den Komplexen *pingens* und *velans* nicht gut. Am Cytoplasma allein liegt das sicher nicht, denn in der (*cruciata* × *biennis*) *rubipicta* vermehren sich die hier nicht ergrünungsfähigen *biennis*-Plastiden ja ausgezeichnet. Vielleicht spielt auch irgend eine Wirkung der kranken Plastiden, die sich gerade in der gegebenen Konstellation von Geno-, Plasto- und Plasmotypus besonders geltend macht, eine Rolle.

Interessanter aber noch ist das Verhalten der kranken Plastiden bei dieser Kreuzung. Die später sterbenden Sämlinge aus *pictiflava* weißrandig × *albivelutina* werden nämlich viel besser grün als die aus Selbstbestäubung gewonnenen. Die defekten Plastiden erzeugen also mit den Kernkombinationen *pingens* · *velans* und *flavens* · *velans* mehr Farbstoff als mit der Kombination *pingens* · M-*flavens*. An den erwachsenen Scheckpflanzen sind die blassen Anteile dann fast so blaß wie bei der albomaculaten M-*pictiflava*. Da den durch Mutation defekt gewordenen Chromatophoren immer noch ein gewisses Vermögen der Farbstoffbildung bleibt, kann es nicht Wunder nehmen, daß die Menge (und Qualität?) der gebildeten Pigmente von der genomatischen Umgebung in ähnlicher Weise abhängig ist wie bei den gesunden Farbträgern. Aber bis jetzt war keine der geprüften Komplexverbindungen imstand, solchen mutierten Plastiden wieder zur Bildung einer Farbstoffmenge zu verhelfen, die die Photosynthese erlaubt hätte.—Daß die Verbindung *flavens* · *flectens* die kranken Plastiden ganz blaß werden läßt, ist nicht verwunderlich, weil schon die gesunden *suaveolens*-Chromatophoren mit dieser Komplexverbindung farblos werden.

#### Belege

- Nr. 18. M-*pictiflava* (aus *cruciata* × MB-*albiflava* albomaculat; 1935/198) 1, weißrandiger Zweig I, selbstb.: 61 weiße, 34 gescheckte bis grüne Sämlinge; ausgepflanzt 32, blühend 14 *pictiflava*, davon 5 gescheckt, 9 grün, und 15 *lutescens*, davon 1 gescheckt, 14 grün. 1936/216.—*Lutescens* kann auftreten, weil der verwendete *flavens*-Komplex sp statt des letalen Sp besaß.
- Nr. 19. Ebenso, weißrandiger Zweig II, selbstb.: 27 weiß, 13 gescheckt bis grün; ausgepflanzt 13, alle blühend, davon 7 *pictiflava*, 5 gescheckt, 2 grün, und 5 *lutescens*, 1 gescheckt, 4 grün. 1936/217.
- Nr. 20. Ebenso, weißrandiger Zweig III, selbstb.: 7 weiße Sämlinge. 1936/226.
- Nr. 21. *pictiflava* 2, weißrandiger Zweig selbstb.: 43 weiß, 2 gescheckt; ausgepflanzt und blühend 2 große gescheckte *pictiflava*. 1936/223.
- Nr. 22. *pictiflava* 3, weißrand. Zweig I selbstb.: 67 weiß, 1 gescheckt, 1 grün; ausgepflanzt und blühend 2 *lutescens*, 1 gescheckt, 1 grün. 1936/231.
- Nr. 23. *pictiflava* 2, ganz weißer Zweig I, selbstb.: 21 weiße. 1936/225. Ebenso, ganz weißer Zweig II selbstb.: 28 weiße. 1936/229.
- Nr. 24. *pictiflava* 2, weißrandiger Zweig × grüner Zweig: 9 weiß, 37 gescheckt, 3 grün; ausgepflanzt 31, meist gescheckt, teils *pictiflava* teils *lutescens*. 1936/224.

- Nr. 25. *pictiflava* 1, ganz weißer Zweig  $\times$  *cruciata*: 2 weiß, 12 gescheckt bis grün; ausgepflanzt 10; blühend 1 *cruciata* gescheckt, 9 *flaviflexa*, 7 gescheckt, 2 grün. 1936/221.
- Nr. 26. *pictiflava* 2, ganz weißer Zweig  $\times$  *cruciata*: 23 weiß, 33 gescheckt, 2 grün; ausgepfl. 31; blühend *cruciata* 7 gescheckt, (davon stark bunt nur 2), 7 grün, und *flaviflexa* 15 gescheckt, davon stark gescheckt 8. 1936/230.
- Nr. 27. *pictiflava* 1, weißrandiger Zweig III  $\times$  *cruciata*: 11 weiß, 25 bunt; ausgepfl. 23; blühend *cruciata* bunt 3, grün 4, und *flaviflexa* bunt 13. 1936/227.
- Nr. 28. *pictiflava* 1, weißrand. Zweig IV  $\times$  *cruciata*: 20 weiß, 15 bunt, 4 grün; ausgepfl. 19; blühend *cruciata* bunt 6, grün 4, und *flaviflexa* bunt 6, grün 2. 1936/228.
- Nr. 29. *pictiflava* 1, weißrand. Zweig I  $\times$  *R-biennis*: 64 blaß, teilweise deutlich hellgrün gescheckt, alle gestorben. 1936/219.
- Nr. 30. *pictiflava* 1, weißrand. Zweig II  $\times$  *R-biennis*: 56 blaß, einige deutlich hellgrün gescheckt, alle gestorben. 1936/222.
- Nr. 31. *pictiflava* 1, weißrand. Zweig II  $\times$  *muricata*: 5 weiß, 29 bunt, 16 grün; ausgepfl. 32, *picticurva* und *flavicurva*, beide teilweise bunt. 1936/220.
- Nr. 32. *pictiflava* 3, weißrand. Zweig I  $\times$  *suaveolens*: 120 blaß, teilweise deutlich hellgrün gescheckt; nur 2 Schecken bleiben am Leben, werden ausgepflanzt, bleiben schwach. 1936/232.
- Nr. 33. *pictiflava* 1, weißrand. Zweig  $\times$  *albivelutina*: 73 gelbgrün, teilweise grünbunt; ausgepfl. 8; blühend 3 *pictivelutina*, 4 *flavivelutina*, alle 7 stark weißbunt. 1936/218.
- Nr. 34. *pictiflava* 3, weißrand. Zweig  $\times$  *rubiflava* mit *muricata*-Plastiden: 17 weiß, 40 bunt, 2 grün; ausgepfl. 21, teils *pingens*  $\cdot$  *flavens*, teils *flavens*  $\cdot$  *ruens*, teils *lutescens* = *flavens*  $\cdot$  *flavens*, fast alle bunt, nur 2 ganz grün. 1936/233.

### Zusammenfassung der Ergebnisse

Bei Artbastarden von *Eu-Oenothera* kommt, wie bei *Onagra*, Variegation des Laubes vor. Die Unterschiede in der Laubfarbe der reziproken Bastarde rühren also mindestens zum Teil von Konstitutionsunterschieden der artspezifischen Plastiden her. Ob die „Erholung“ der in  $F_1$  blassen Bastarde in späteren Generationen (SCHWEMMLE) mit der Aussortierung der besser ergrünenden Plastiden zusammenhängt, bleibt zu untersuchen.

Besseres Ergrünen blasser *Onagra*-Bastarde in der zweiten Generation ist wieder nicht beobachtet worden, wenn nur eine einzige Plastidensorte vorhanden ist. Die Beobachtung soll aber über eine größere Zahl von Generationen fortgesetzt werden.

Für das Erhaltenbleiben der artspezifischen Qualität der Plastiden in Bastarden wird neues Material beigebracht.

Die Vermehrungsgeschwindigkeit der Plastiden scheint wie ihre Ergrünungsfähigkeit von der idiotypischen, vor allem genotypischen Umgebung abzuhängen.

Auch durch Mutation defekt gewordene Plastiden erzeugen in Verbindung mit verschiedenen Komplexkombinationen verschiedene Mengen (und Qualitäten?) von Farbstoff, wie die gesunden Plastiden.

Partenkirchen, im September 1936

### Zitierte Literatur

- Renner, O., Zur Kenntnis der nichtmendelnden Buntheit der Laubblätter. *Flora* 130, 218–290 (1936).
- Schwemmler, J., Die Rolle des Plasmas für die Vererbung. *Der Erbarzt* 1, 179–185 (1935).

## Possibility of Crossing-over between Semihomologous Chromosomes from Two Different Genoms<sup>1)</sup>

By

H. Kihara and I. Nishiyama

Kyoto Imperial University

It is remarkable that the artificially raised allopolyploids show high irregularities in maturation divisions. They are often highly sterile. For instance, *Aegilotriticum* obtained in our Laboratory (KIHARA and KATAYAMA 1931) had often 2 univalents in the first maturation division, while they are rarely found in the parents. The fertility was 39% by selfing and 61% in open pollination. Correspondingly gametes with 20 (or 22) chromosomes, besides normal ones, were expected. The occurrence of the 20-chromosome gamete was proved by the crossing experiments. Abnormality is more frequent in *Secalotriticum* studied by LEWITSKY and BENETZKAIA (1931). 2-6 or even more univalents were found during the first maturation division. Abnormalities do exist also in the maturation divisions of the natural allopolyploids. But they are rather rare.

The reason why the abnormalities in the maturation divisions of newly produced allopolyploids are very often, is not sufficiently investigated.

Since 1934 we have had the opportunity to study many individuals obtained from the back-crossing, (*Triticum polonicum* × *Haynaldia villosa*) × *T. polonicum* which showed great diversity in the intensity of the chromosome conjugation and number of bivalent chromosomes.

The authors have also given attention to the origin of gametes having eu-, hypo-, and hypertriploid chromosomes. Genetical studies which are not yet completed, will be published in another paper.

### Material and methods

*Haynaldia villosa* ( $n = 7$ ) was sent by Dr. J. MOTTE (Montpellier), to whom we express our sincere thanks. *Triticum polonicum* ( $n = 14$ ) is the old material in our culture since 1919 (KIHARA 1924).

One  $F_1$  (*T. polonicum* × *H. villosa*) was made by Mr. WAKAKUWA in 1934. This was back-crossed with *T. polonicum*, *dicoccum*

1) Contributions from the Laboratory of Genetics, Biological Institute, Kyoto Imperial University, No. 82.

and *spelta*. The back-cross gave many plants. The symbol for  $F_1$  obtained from the back-crossing is  $BF_1$ . Two  $BF_1$  ( $F_1 \times polonicum$ ) were again back-crossed with *T. polonicum*. The results of crossing experiments are given in Table 1.

Table 1. Results of crossing experiments

♀	♂	No. of florets	No. of seeds obtained (%)	No. of plants obtained (%)
<i>T. pol.</i> × <i>H. vill.</i>		120	1(0.8)	1(0.8)
$F_1$ × <i>T. polonicum</i>		192	66(34.4)	33(17.2)
$F_1$ × <i>T. dicoccum</i>		150	39(26.0)	34(22.7)
$F_1$ × <i>T. spelta</i>		50	15(30.0)	13(26.0)
Sum		392	120(30.6)	80(20.4)
$BF_1-20$ × <i>T. polonicum</i>		24	7(29.1)	6(25.0)
<i>T. pol.</i> × $BF_1-14$		30	5(16.7)	3(10.0)

For the cytological study, permanent and smeared preparations are employed as usual.

The chromosome numbers of the  $F_1$  and its offspring were determined by chromosome counts of PMCs. Sometimes the results were ascertained by the microscopical investigations on root tips.

### Meiosis in $F_1$

As expected, the  $F_1$  had 21 chromosomes. Bivalent chromosomes in the first metaphase of PMCs are almost always very weakly associated (Figs. 1-3). Their number varies from 0-4. Bivalents with one interstitial chiasma are sometimes found (Fig. 4). Counts of 100 PMCs are given in Table 2.

The number of univalents varies from 13 to 21. They assemble in the early metaphase usually near



Figs. 1-4. PMCs at the first metaphase in *T. polonicum* × *H. villosa*  $F_1$  ( $\times 1632$ ). 1. 21 univalents. 2. 1 bivalent and 19 univalents. 3. 2 bivalents and 17 univalents. 4. 4 bivalents and 13 univalents.

Table 2. Variation of bivalent chromosomes in PMCs of the  $F_1$  (*T. polonicum*  $\times$  *H. villosa*)

No. of Biv.	0	1	2	3	Total
Frequency	42	38	18	2	100

the poles, before they move toward the equator. They form often very regular nuclear plates. In spite of the regular arrangement of the univalents only a part

of them are divided equationally in the anaphase and the undivided build often a chromosome bridge.

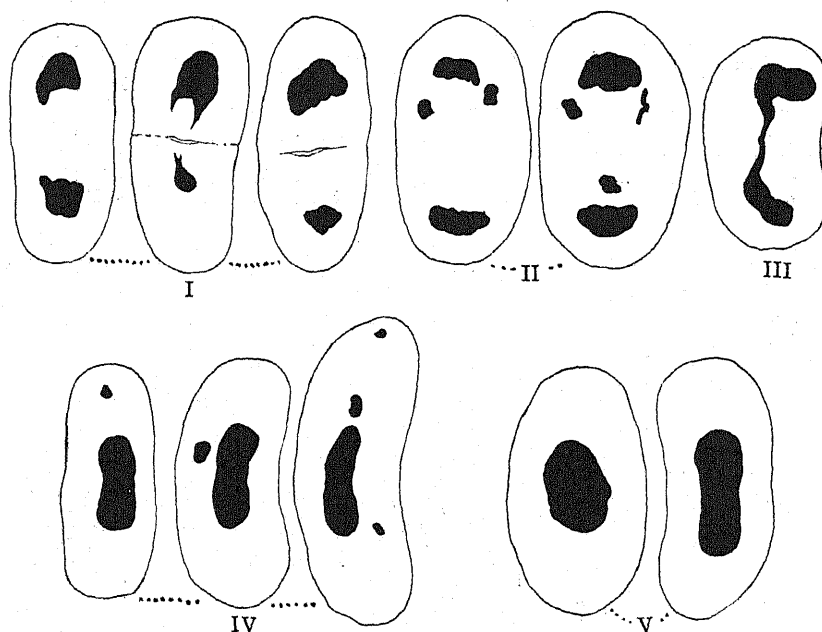


Fig. 5. PMCs at the first telophase ( $\times 1360$ ). I. Chromosomes are divided into two daughter nuclei. II. The same with small additional micronuclei. III. Two daughter nuclei are bound by a chromosome bridge. IV. One restitution nucleus with small isolated nuclei. V. One big restitution nucleus.

The chromosome bridge seems, in most cases, to be cut into two pieces during the cytokinesis. Often the chromosomes arranged near the equator do not move to the poles and are included in a giant restitution nucleus.<sup>1)</sup> The PMCs in the telophase are classified into the following 5 types (Fig. 5).

- I. Two daughter nuclei are present.
- II. Several micronuclei are present besides two daughter nuclei.
- III. Daughter nuclei are bound with a chromosome bridge.

1) On the behaviour of chromosomes in hybrids see "Genomanalyse II" (KIHARA 1931).

IV. One large nucleus (principal nucleus) and 1-2 or more micronuclei.

V. One large restitution nucleus.

The frequency of these 5 types of PMCs is given in Table 3. The tetrad formation is accordingly somewhat complicated.

Table 3. Frequency of different types of interkinesis PMCs as shown in Fig. 5.

Types	I	II	III	IV	V	Total
Frequency (%)	18 17.0	47 44.3	10 9.4	9 8.5	22 20.8	106 100

The gametes having 21 chromosomes (triploid), are originated from PMCs belonging to the type V. The hypo- and hypertriploid gametes can be produced from PMCs of the type IV. The assumption is based on the behavior of aberrant dyad chromosomes included in micronuclei. It is known that the micronuclei often show no indication of mitosis and are very often eliminated in the cytoplasm (KIHARA and LILIENFELD 1936). Owing to the loss of the chromosome or chromosomes the principal nucleus gives hypotriploid daughter one. However, if they are included without division in one of the daughter halves of a principal nucleus, they may have hypertriploid number.

### Chromosomes of back-crossed $F_1$ ( $BF_1$ )

$BF_1$  individuals had 34-35 chromosomes, when *T. polonicum* was used as pollen parent. The functional female gametes contained, in these cases, 20-21 chromosomes. But the variation of chromosome number was great, when *T. dicoccum* ( $n = 14$ ) and *T. spelta* ( $n = 21$ ) were used as pollen plants. The results are shown in Table 4.

Table 4. Frequency of functional gametes with different-chromosome numbers. Results obtained from back-crosses

Chromosome number	18	19	20	21	22	23	Total
$F_1 \times \textit{polonicum}$	0	0	3	11	0	0	14
$F_1 \times \textit{dicoccum}$	1	1	2	3	0	1	8
$F_1 \times \textit{spelta}$	0	1	2	0	2	1	6
Total	1	2	7	14	2	2	28

It is clear that the gametes with 21 chromosomes are most frequently fertilized in the back-crosses and 20-chromosome gametes are also often fertilized.

The chromosome conjugation in the PMCs was very carefully studied in  $BF_1$  with *T. polonicum* and *T. dicoccum*. The results obtained from  $F_1 \times T. polonicum$  were summarized in Table 5.

From this table (Tab. 5) we can see at a glance that the conjugation of bivalents, even in plants with 35 chromosomes, is not always quite as normal as expected. The gametes with 21 chromo-



Table 5. Chromosome conjugation in PMCs and fertility of BF<sub>1</sub> (F<sub>1</sub> × *polonicum*)

Plant No.	2n	Conjugation (Modification)	Tri- valent	Tetra- valent	Pollen grains	Fertility %	
						Open	Self
3	35	14 <sub>II</sub> +7 <sub>I</sub> , (15 <sub>II</sub> +5 <sub>I</sub> )	++	+	bad	1.4	—
4	35	14 <sub>II</sub> +7 <sub>I</sub>	+	no N	very good	16.1	6.6
5	34	13 <sub>II</sub> +8 <sub>I</sub>	++	++	bad	0.0	—
9	35	14 <sub>II</sub> +7 <sub>I</sub>	+	++	good	1.9	0.0
12	34	13 <sub>II</sub> +8 <sub>I</sub> (14 <sub>II</sub> +6 <sub>I</sub> )	(no complex)	++	bad	5.0	—
14	(35)	—	—	—	very good	19.8	7.7
19	35	14 <sub>II</sub> +7 <sub>I</sub> (13 <sub>II</sub> +9 <sub>I</sub> )	+	++	less good	1.9	0.0
20	35	14 <sub>II</sub> +7 <sub>I</sub>	—	—	very good	19.4	—
21	35	14 <sub>II</sub> +7 <sub>I</sub> , 15 <sub>II</sub> +5 <sub>I</sub>	++	++	—	0.0	—
23	35	14 <sub>II</sub> +7 <sub>I</sub>	no complex found		very good	22.2	13.9
24	35	14 <sub>II</sub> +7 <sub>I</sub>	no complex found		very good	22.4	9.5
25	35	13 <sub>II</sub> +9 <sub>I</sub> (14 <sub>II</sub> +7 <sub>I</sub> )	++	++	good	4.4	0.0
26	34	13 <sub>II</sub> +8 <sub>I</sub>	++	++	less good	2.7	—
32	35	14 <sub>II</sub> +7 <sub>II</sub>	+	+	very good	—	25.0

++ one trivalent (tetravalent) is often found

+ rarely found

— not examined

somes should have 14 *polonicum* and 7 *villosa* chromosomes. Therefore it may easily be assumed that the conjugation between 14 *polonicum* chromosomes from parents occurs quite normally. This is true for plants Nos. 4, 20, 23, 24 (Figs. 9–10) and 32, but not for plants Nos. 3, 9, 19 and 21 (Figs. 6–8).

The studies are made on the number of cells with trivalent and tetravalent chromosome complexes in a BF<sub>1</sub> plant (BF<sub>1</sub>-21). The results are given in Tab. 6.

Table 6. Frequency of the number of PMCs with trivalent and tetravalent complexes in BF<sub>1</sub>-21

Prep. No.	PMCs with no complex	PMCs with		PMCs with 1 <sub>IV</sub>					PMCs with 1 <sub>IV</sub> +1 <sub>III</sub>		Total
		1 <sub>III</sub>	2 <sub>III</sub>						2 <sub>IV</sub>	1 <sub>IV</sub> +1 <sub>III</sub>	
3509-2	53	19	9	2	2	18	3	2	3	1	114
3509-4	11	3	3	0	2	1	1	0	0	0	21
Total	64	22	12	2	4	19	4	2	3	1	135
	64	36	4			29			2		135

The complexes were found in nearly 50% of PMCs. The number of paired chromosomes is 14 or 15, if we count a trivalent as a single pair and a tetravalent as two.

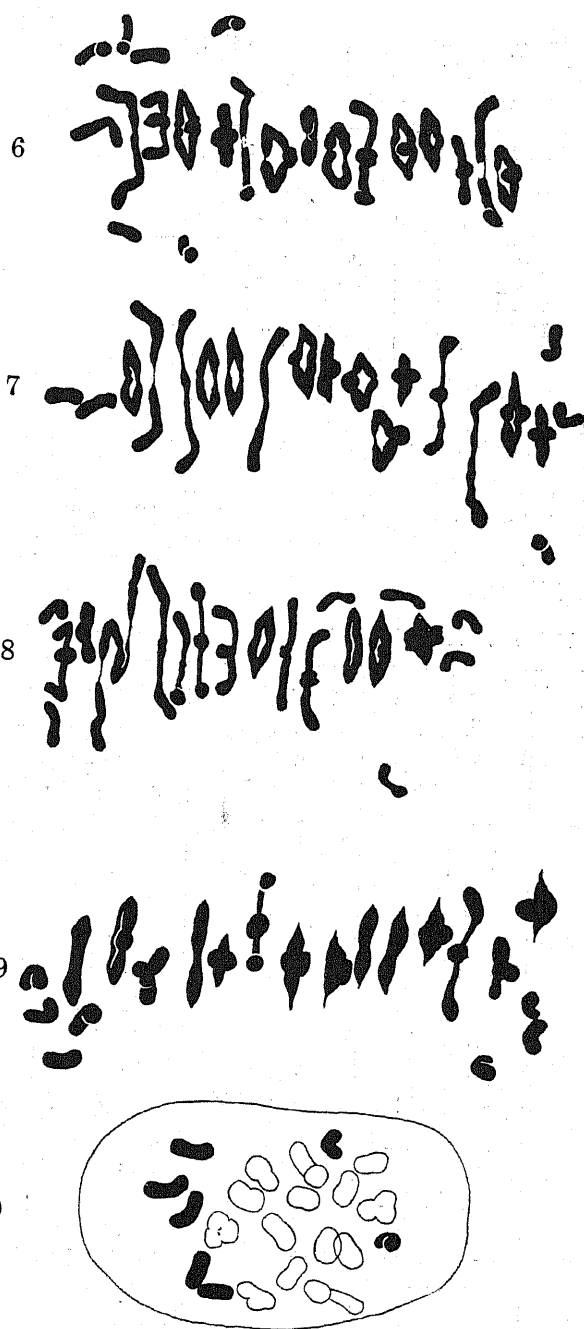
Plants with 34 chromosomes have almost always a chromosome formula 13<sub>II</sub>+8<sub>I</sub>; tri- or tetravalent complex was found in most cases. One of them, which has often the modified conjugation 14<sub>II</sub>+6<sub>I</sub>, forms no complex.

The fertility of the  $BF_1$  plants is high, if there is no complex, and the combination of chromosomes is almost invariably  $14_{II} + 7_I$ . The plants with complexes gave no offspring by selfing.

In  $F_1 \times dicoccum$  the formation of trivalents and tetravalents was very frequent. In one plant (No. 6)  $(1-3)_{III}$  and  $(1-2)_{IV}$  were found. The reason can be ascribed to the slight difference of AB genomes between *polonicum* and *dicoccum* (cf. HOSONO, 1935). Therefore we have not used this material for further investigations.

#### $BF_1 \times T. polonicum$ and $BF_2$

For the back-crossing of  $BF_1$  ( $F_1 \times polonicum$ ), two individuals,  $BF_1-14$  and  $-20$  were used. The former had presumably  $14_{II} + 7_I$ . The latter showed  $14_{II} + 7_I$  clearly in PMCs (Tab. 5).



Figs. 6-10. Chromosomes at the first metaphase of two plants obtained from the back-cross,  $F_1 \times T. polonicum$  ( $\times 2067$ ). 6-8. Chromosomes of  $BF_1-21$ . 6.  $14_{II} + 7_I$ . 7.  $15_{II} + 5_I$ . 8.  $1_{IV} + 12_{II} + 7_I$ . 9-10. Chromosomes of  $BF_1-24$  showing  $14_{II} + 7_I$ . 9. side view. 10. Polar view.

Table 7. Chromosomes and fertility in individuals obtained from *T. polonicum* × BF<sub>1</sub>-14 (185) and BF<sub>1</sub>-20 × *polonicum* (187)

Plant No.	2n	n	Fertility (%)
185-1	28	14 <sub>II</sub>	86.5 (open)
185-3	28	14 <sub>II</sub>	75.0 (self)
187-1	28	14 <sub>II</sub>	86.8 (open)
187-2	33	14 <sub>II</sub> +5 <sub>I</sub>	2.0 (open)
187-4	35	14 <sub>II</sub> +7 <sub>I</sub>	7.1 (self)
187-5	35	14 <sub>II</sub> +7 <sub>I</sub>	22.9 (open)

also identical to *T. polonicum*. They have no *villosa*-hairs on the leaves. However, one individual (185-3) had shorter culms and blumed 2 weeks later. Its fertility (75%) is not very low, if we consider the bagged condition. Whether or not this plant (185-3) has obtained a gene block from the *villosa*-genom is so far unknown.

Many BF<sub>2</sub> plants were studied karyologically. But only a part of them are shown in Tab. 8.

Table 8. Variation in the chromosome number and the conjugation in PMCs of BF<sub>2</sub>

Plant No.		2n	Conjugation	Modification	Remarks	Fertility (Self) (%)
BF <sub>1</sub>	BF <sub>2</sub>					
4	193-2	34	15 <sub>II</sub> +3 <sub>I</sub> +1fr(?)	often 14 <sub>II</sub> +5 <sub>I</sub> +1fr	often N- and ∞- complex	1.6
	-3	33+1fr	15 <sub>II</sub> +3 <sub>I</sub> +1fr			—
14	203-4	37	15 <sub>II</sub> +7 <sub>I</sub>	9 <sub>I</sub>	1 <sub>III</sub> , 1 <sub>IV</sub> 1 heteromor- phic pair probably 1 frag. 2 <sub>III</sub>	8.3
	-9	34	16 <sub>II</sub> +2 <sub>I</sub>	4 <sub>I</sub>		0.0
	-10	37	16 <sub>II</sub> +5 <sub>I</sub>	7 <sub>I</sub>		2.3
	-11	37	1 <sub>III</sub> +17 <sub>II</sub>	5 <sub>I</sub>		0.0
	-12	36	18 <sub>II</sub> +1 <sub>I</sub>	1 <sub>III</sub> +15 <sub>II</sub> +3 <sub>I</sub> , (6 <sub>I</sub> )		1.2
	-13	40	16 <sub>II</sub> +4 <sub>I</sub> 17 <sub>II</sub> +6 <sub>I</sub>	1 <sub>III</sub> +17 <sub>II</sub> +3 <sub>I</sub> 18 <sub>II</sub> +4 <sub>I</sub>		5.7
23	213-2	37	15 <sub>II</sub> +7 <sub>I</sub>	16 <sub>II</sub> +5 <sub>I</sub> 17 <sub>II</sub> +3 <sub>I</sub> (rare) 14 <sub>II</sub> +7 <sub>I</sub> (rare)		2.9
	-7	35	15 <sub>II</sub> +5 <sub>I</sub>	14 <sub>II</sub> +7 <sub>I</sub> +1fr		1.3
	-12	35+1fr	13 <sub>II</sub> +9 <sub>I</sub> +1fr	15 <sub>II</sub> +5 <sub>I</sub> +1fr		0.0
24	214-1	36+2fr	17 <sub>II</sub> +3 <sub>I</sub> +1fr	14 <sub>II</sub> +8 <sub>I</sub> +2fr 16 <sub>II</sub> +5 <sub>I</sub> +1fr	1 heteromor- phic pair	0.0
	-13	38+1fr	15 <sub>II</sub> +8 <sub>I</sub> +1fr	1 <sub>III</sub> +15 <sub>II</sub> +5 <sub>I</sub> +1fr 17 <sub>II</sub> +4 <sub>I</sub> +1fr		—
32	221-2	37	17 <sub>II</sub> +3 <sub>I</sub>	1 <sub>III</sub> +16 <sub>II</sub> +2 <sub>I</sub> 1 <sub>IV</sub> +14 <sub>II</sub> +5 <sub>I</sub>	1 heteromorphic pair (sometimes isolated) 1 <sub>III</sub> , rarely 1 <sub>IV</sub> rarely N- complex	0.8
	-6	37	17 <sub>II</sub> +3 <sub>I</sub>	16 <sub>II</sub> +5 <sub>I</sub>		7.9
	-9	37	16 <sub>II</sub> +5 <sub>I</sub>	1 <sub>III</sub> +16 <sub>II</sub> +2 <sub>I</sub> 1 <sub>III</sub> +14 <sub>II</sub> +6 <sub>I</sub>		15.2
	-11	33	14 <sub>II</sub> +5 <sub>I</sub>	1 <sub>III</sub> +15 <sub>II</sub> +4 <sub>I</sub>		15.0
			1 <sub>III</sub> +13 <sub>II</sub> +4 <sub>I</sub>			

The results of crossing experiments are quite similar to those obtained from back-crossing pentaploid wheat hybrids with 4x parent (Tab. 7).

The conjugation of chromosomes is very tight and regular. The fertility of these plants is normal, if the chromosome number is 28 as *T. polonicum*. The morphological characters are

The variation in chromosome number is quite similar to that of the pentaploid wheat hybrids. But the conjugation of chromosomes is more complicated than that of  $BF_1$ . However the  $BF_2$ , which had no trivalent or tetravalent complex in  $BF_1$  (e.g. Plant Nos. 23 and 24), have again no complex. The fluctuation of the number of bivalents within one and the same individual is very large. For instance  $BF_2$  213-2 has 15-17 bivalents.

It is significant that one or two fragments are often found. The reason can be traced back to the second division in the  $BF_1$ , where very often the lagging chromosomes are fragmented (Fig. 11).

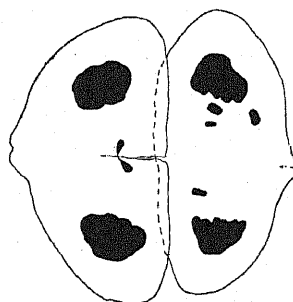


Fig. 11. Second division of  $BF_1$ -24 showing fragmentation of a lagging chromosome.

### Discussion

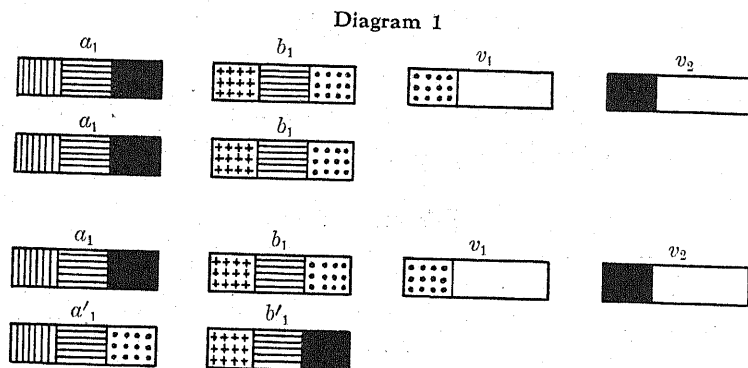
The studies of the hybrid *T. polonicum*  $\times$  *Haynaldia villosa* showed that 0-4 bivalents were found in the first maturation division. We know already that the maximum intergenomic conjugation between chromosomes of A and B is 3 (KIHARA and NISHIYAMA, 1930). Therefore the fourth bivalent must be derived from the allosyndesis between semihomologous chromosomes of A or B and V. In the hybrid *T. aegilopoides*  $\times$  *H. villosa*, however, 0-4 bivalents were found (KIHARA unpublished). The mode was 2. This indicates that at most 4 semihomologous partners exist between  $A_{Eink.}$  and V. The genom A of Einkorn and A of Emmer are not true homologous, as already indicated by KIHARA and LILIENFELD (1932). But the two are practically homologous. Therefore the number of bivalents in ABV can still be higher. At least we might find PMCs with a maximum of 5 bivalents, if we use a large enough number of PMCs.

In  $BF_1$  we found individuals quite different in their mode of chromosome conjugation; namely some of them were almost constant in the chromosome combination showing  $14_{II} + 7_I$ . The remaining ones have also  $14_{II} + 7_I$ , but the trivalent and tetravalent complex are seen in different frequency and in two individuals nuclear plates with  $15_{II} + 5_I$  and  $14_{II} + 7_I$  were found. One of them ( $BF_1$ -21) had these two modes of chromosome conjugation in an equal frequency.

The unreduced gametes of  $F_1$  have a priori 14 *polonicum*- and 7 *villosa*-chromosomes. Therefore we can expect only one chromosome combination, namely  $14_{II} + 7_I$  or its derivative,  $13_{II} + 9_I$ . But if 1-2 *villosa*-chromosomes associate with two corresponding ones from AB, we might find  $1_{III} + 13_{II} + 6_I$  and  $2_{III} + 12_{II} + 5_I$ . However

$15_{II} + 5_I$  were found in about 50% of all PMCs in BF<sub>1</sub>-21. This indicates undoubtedly that changes occurred in the *polonicum* chromosome complement derived from F<sub>1</sub>.

14 *polonicum*-chromosomes in pure species, conjugate quite normally with their partners and form 14 bivalents. Only in very rare cases can one find 1-2 univalents. Tetravalents were not found (HOSONO 1935). Therefore the formation of chromosome complexes in BF<sub>1</sub>-21 can be explained by the structural change in one of the *polonicum*-chromosomes. Such a change can be demonstrated in Diagram 1.



In this diagram  $a_1$  and  $b_1$  are chromosomes from A- and B-genoms respectively. They are homologous in the middle part. The crossing-over took place in this part.  $a'_1$  and  $b'_1$  are chromosomes thus made.  $v_1$  and  $v_2$  are *villosa*-chromosomes which are homologous at their end to  $a_1$  and  $b_1$  respectively. If the 12 remaining chromosomes have no change in structure, then  $12_{II} + a_1 a'_1 + b'_1 v_2 + b_1 v_1$  make  $15_{II}$ . Two of them,  $b'_1 v_2$  and  $b_1 v_1$  must associate loosely. If this holds true, we might expect a circle of 4 chromosomes ( $a_1 a'_1 b_1 b'_1$ ), an N-complex ( $v_1 b_1 b'_1 v_2$ , or  $v_1 a_1 a'_1 v_2$ ), two v-shaped trivalents ( $a_1 a'_1 v_1$  and  $b_1 b'_1 v_2$ ) and two Y-shaped trivalents ( $a'_1 b_1 v_1$  and  $a_1 b'_1 v_2$ ). All of these complexes are found in reality. But neither the penta- nor the hexavalent complex which may occur in a very rare occasion, was so far observed. However two tetravalents in one cell, which were found in 135 PMCs (Tab. 6), cannot be explained by this hypothesis. One more structural change in *polonicum*-chromosome will suffice for the requirement. But it may be unnecessary and perhaps impossible to give accurate illustrations for all figures observed. We will be satisfied, if a possible way for the formation of a complex is given. It may be mentioned here that the force of chromosome conjugation may be weakened, or sometimes strengthened within a limited scope by the structural change in chromosomes

and the occurrence of univalents. If such phenomena occur really in our present case, the supernumerary tetravalents can be explained by the realization of the otherwise latent affinity between two pairs of A and B genoms. The reduction of the number of the bivalent chromosomes may also be explained in a similar way.

The explanation for the formation of  $13_{II} + 8_I$  and  $14_{II} + 6_I$  is rather easy. The hypotriploid gametes have lost chromosome "a<sub>1</sub>" of *polonicum*, then  $13_{II} + a_1 + v_{1-7} = 13_{II} + 8_I$ . As  $a_1$  and  $v_2$  are semihomologous with each other sometimes  $13_{II} + a_1v_2 + 6_I$  should be made. If the lost chromosome is not homologous to one of the seven *villosa*-chromosomes, they have only one combination  $13_{II} + 8_I$  (e.g. BF<sub>1</sub>-5).

In the main, the working hypothesis seems to be in harmony with results obtained in BF<sub>1</sub>. But we do not intend to explain the whole mechanism of the unexpected chromosome conjugation with the simplest form of structural change, i.e. a single crossing-over between semihomologous chromosomes. The change is hereditary as the investigations with BF<sub>2</sub> clearly indicate.

This conclusion seems to be justified further by the results from back-crossing of BF<sub>1</sub> ( $14_{II} + 7_I$ ) to *T. polonicum*. The conjugation of bivalents is very regular in all plants obtained from this crossing (Tab. 7).

The genomtype of BF<sub>1</sub> is AABBV and that of F<sub>1</sub> is ABV. The crossing-over between semihomologous partners from A and B may easily occur only when A and B have no true homologous genoms, namely in ABV. The occurrence of an interstitial chiasma is also favorable for this assumption (Fig. 6. See also KIHARA, 1936). KOSTOFF (1936) has illustrated the possibility of the crossing-over between semihomologous partners.

The fluctuation of the number of bivalent chromosomes in BF<sub>2</sub> (No. 213-2) must be considered here. It is generally accepted that the pairing of homologous chromosomes is disturbed if there are many unpaired chromosomes. The pentaploid wheat hybrid with  $14_{II} + 7_I$ , for instance, has often  $13_{II} + 9_I$  and in rare cases  $12_{II} + 11_I$ . The BF<sub>2</sub> 213-2 has 15-17 bivalents and 7-3 univalents. If we accept the above principle and assume that there are 17 homologous pairs and 3 nonhomologous chromosomes, the isolation of homologous partners are too frequent. The fertility of the plant is also too high (2.9%), as this plant is assumed to belong to the group of sterile chromosome combination (KIHARA 1924). We can not yet decide whether the principle under consideration may be applied in this case or in the change of chromosome complements which had already occurred in the 17 pairs in the preceeding generation. The latter

alternative seems to fit this case. The change is perhaps due to the crossing-over between partners from A (or B) and V.

The fertility of 35-chromosome  $BF_1$  individuals is high, when the bivalents are closely associated and no complex is found in the first maturation division. The fertility of  $BF_1 \times T. polonicum$  showing  $14_{II} + 7_I$  is equally as high as that of  $BF_1$  plants ( $14_{II} + 7_I$ ). On the other hand the fertility of 35-chromosome plants with a trivalent or tetravalent complex is very low, and in one case completely sterile. The low fertility can be explained by the incompleteness of the concerned genom, as in the case of a single segmental interchange (KIHARA and LILIENFELD 1932).

In this connection it is very important to note that the artificial allopolyploids produced by temperature is also highly sterile (DORSEY 1936). It is assumed that chromosome doubling took place just after the fertilization probably by the corruption of the first mitosis. The structural change of chromosomes is hardly possible. Cytological investigation of these hybrids is necessary.

Many authors (TSCHERMAK 1934, v. BERG 1935, SANDO 1935 and KOSTOFF 1936) have recently produced fertile  $F_2$  in similar crosses. According to SANDO, the  $F_2$  plants of  $T. turgidum \times H. villosa$  produced an average seed set of 29.7%, with a maximum of 58.8% for a single plant. The  $F_3$  was much higher in fertility. They produced an average seed set of 58.5%, with a maximum of 76.9% for a single plant. Although the chromosomes were not studied, the  $F_2$  and  $F_3$  might be hexaploid. The individual difference in fertility can be explained by the difference in chromosomal abnormalities caused by the structural change.

*T. turgidovillosum* reported by v. BERG (1935) has shown relatively normal conjugation giving in most cases  $21_{II}$ . Correspondingly the fertility seems to be very good. He observed that in  $F_1$  hybrids the 21 univalent chromosomes divide longitudinally in the first maturation division and give unreduced gametes, owing to the regression of the second division. In our present material, the longitudinal splitting of all univalents seems to be very rare. We could not find such phenomena in our material. On the contrary the restitution nuclei caused by the regression were found very frequently. The percentage of such PMCs is 20.8% (Tab. 3).

The fertility of  $F_1$  obtained by the back-cross with *T. polonicum* and *T. dicoccum* and *T. spelta* is 20.4%. As functional gametes contain also hypo- and hypertriploid chromosomes, PMCs of the category IV, must give also functional gametes. The sum of the two types, IV and V, is 29.3%. If we take into consideration the technical difficulties in pollination and mortality of gametes derived from

PMCs of IV, the difference between the calculated fertility and the result obtained may not be significant.

v. BERG assumed in general that the unreduced gametes may arise in different ways. He gave (1) regression of the first division, (2) fusion of two sister plates in the second division and (3) equational division of all univalents in the first maturation division following the regression of second metaphase etc. He assumed that the last one is most probable in his case. After v. BERG the last process is also suitable for the formation of gametes with pseudosomatic chromosome number.<sup>1)</sup> From our observations the gametes with pseudosomatic chromosome number may be also produced from PMCs with one large regression nucleus and two micronuclei (dyads). If one of the micronuclei (one dyad) is lost in the plasm, and the other is included in the daughter halves of the principal nucleus, the gametes with pseudosomatic chromosome number will be formed. As already shown, we can easily explain the formation of hyper- and hypotriploid gametes from similar mechanism.

The microscopical observations made by v. BERG were closely alike in many respects except the longitudinal division of all univalents. The difference lies perhaps in the mother plants (*T. turgidum* and *T. polonicum*).

The significance of gametes with so-called pseudosomatic chromosome number, for the complex formation in  $BF_1$  may briefly be touched. Assume, that a gamete with 21 chromosomes lacks chromosome "a" and has two "b" chromosomes instead, and the remaining 19 chromosomes are normal. If such a female gamete is fertilized by the normal *polonicum*-sperm, we get a 35-chromosome individual. The conjugation must be theoretically  $1_{III} + 12_{II} + 8_I$  or  $13_{II} + 9_I$ .

If the loss and doubling of chromosomes occur within *villosa*-genom, we can expect the chromosome conjugation  $15_{II} + 5_I$ . In this case, however, we cannot expect frequent occurrence of complexes, even if the duplicated chromosomes be the semihomologous one (for instance  $v_1$  shown in Diagram 1). So far as the change of chromosome number in the offspring of AABBV is concerned, its behavior is quite similar to that of the pentaploid wheat hybrids (KIYAHARA 1924) and triploid *Avena* hybrids (NISHIYAMA 1934). The somatic chromosome number varies from 33 to 40. The plants belonging to the diminishing chromosome group will go to the *polonicum* condition (AABB), and those of the increasing group will go to the final

1) Chromosome number of the gametes is 21, but the content is not the same as the unreduced ones. Such gametes fail to have one chromosome from a complete complement (ABV), one chromosome exists in double dose. Such can be produced from PMCs with  $1_{II} + 19_I$ .



allopolyploid condition (AABBVV). In these conditions the plants will recover complete fertility, if there is no other cause to disturb the physiological balance.

From these considerations made in the preceding lines we may conclude, in general, that the structural change in the chromosome can be a cause of the abnormality in the maturation divisions and low fertility of allopolyploids.

#### Literature

- v. Berg, K. H. 1935. Cytologische Untersuchungen an den Bastarden des *Triticum turgidovillosum* und an einer  $F_1$  *Triticum turgidum*  $\times$  *villosum*. (Weitere Studien am fertilen konstanten Artbastard *Triticum turgidovillosum* und seinen Verwandten) III. Teil. Zeitschr. Indukt. Abst.- u. Vererbungsl. 68.
- Dorsey, E. 1936. Induced polyploidy in wheat and rye. Chromosome doubling in *Triticum*, *Secale* and *Triticum-Secale* hybrids produced by temperature changes. Journ. Hered. 27.
- Hosono, S. 1935. Karyogenetische Studien bei reinen Arten und Bastarden der Emmerreihe. I. Reifungsteilungen. Japan. Journ. Bot. 7.
- Kihara, H. 1924. Cytologische und genetische Studien bei wichtigen Getreidearten mit besonderer Rücksicht auf das Verhalten der Chromosomen und Sterilität in den Bastarden. Mem. Coll. Sci. Kyoto Imp. Univ. B. 1.
- 1931. *Aegilotriticum* und *Aegilops cylindrica*. (KIHARA, H. Genomanalyse bei *Triticum* und *Aegilops*. II). Cytologia 2.
- 1936. Ein diplo-haploides Zwillingsspaar bei *Triticum durum*. (Japanisch mit deutscher Zusammenfassung). Agric. and Hortic. 11.
- und Katayama, Y. 1931. Zur Entstehungsweise eines neunten konstanten oktaploiden *Aegilotriticum* (KIHARA, H. Genomanalyse bei *Triticum* und *Aegilops* III). Cytologia 2.
- und Lilienfeld, F. 1932. Untersuchungen an *Aegilops*  $\times$  *Triticum*- und *Aegilops*  $\times$  *Aegilops*-Bastarden (KIHARA, H. Genomanalyse bei *Triticum* und *Aegilops* IV). Cytologia 3.
- und — 1936. Riesenpollenkörner bei den  $F_1$ -Bastarden *Aegilops squarrosa*  $\times$  *Haynaldia villosa* und *Aegilops caudata*  $\times$  *Aegilops speltoides*. Japan. Journ. Gen. 12.
- und Nishiyama, I. 1930. Genomaffinitäten in tri-, tetra-, und pentaploiden Weizenbastarden. (KIHARA, H. Genomanalyse bei *Triticum* und *Aegilops*. I). Cytologia 1.
- Kostoff, D. 1936. Studies on polyploid plants. Polyploid forms in *Triticum* experimentally produced XII. Bull. Acad. Sci. USSR.
- Lewitsky, G. A. and Benetzkaja, G. K. 1931. Cytology of the wheat-rye amphidiploids. Bull. Appl. Bot. Vol. 27 No. 1.
- Nishiyama, I. 1934. Chromosome behavior and its bearing on inheritance in triploid *Avena* hybrids. Mem. Coll. Agric., Kyoto Imp. Univ. 32.
- Sando, W. J. 1935. Intergeneric hybrids of *Triticum* and *Secale* with *Haynaldia villosa*. Journ. Agric. Res. 51.
- Tschermak-Seysenegg, E. 1934. Weitere Studien am fertilen, konstanten *Tr. turgidovillosum* und seinen Verwandten. I. Teil. Zeitschr. Indukt. Abst.- u. Vererbungsl. 66.

# **SAT-Chromosom und Spiralstruktur der Chromosomen der extrakapsulären Körper (*Merodinium spec.*) von *Collozoum inerme* Müller**

Von

**K. Patau**

Aus dem Kaiser-Wilhelm-Institut für Biologie, Berlin-Dahlem,  
Abt. Hartmann und der Zoologischen Station, Neapel

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## **I. Einleitung**

Die Kernteilungen der von *Collozoum* und *Sphaerouzoum* bekannten extrakapsulären Körper gehen wie die intrakapsulär ablaufenden Mitosen des gleichen Typus, die sich bei diesen und anderen Radiolarien finden, der Bildung sog. Anisosporen voraus. Die Natur dieser Anisosporen ist umstritten. Wie ich an anderer Stelle ausführen werde, sehe ich in dem Vergleich der niedrigen Chromosomenzahl der Anisosporen mit den offenbar weit mehr als doppelt so großen Zahlen der vegetativen Teilungen ein schwerwiegendes Argument (auf das bereits CHATTON kurz hingewiesen hat) zugunsten der vor allem von diesem Autor (zuletzt 1934) vertretenen Auffassung, wonach die Anisosporen garnicht in den Generationskreis der Radiolarien gehören, sondern parasitische, mit *Syndinium* nahe verwandte Peridineen darstellen, für die CHATTON (1923) die Gattung *Merodinium* gründete. Die Frage, ob die extrakapsulären Körper zu *Merodinium Brandti* CHATTON, das sich in *Collozoum inerme* intrakapsulär entwickelt, zu rechnen sind oder eine eigene Art darstellen, muß z. Zt. offenbleiben.

Die bereits von HARTMANN und HAMMER (1909) kurz gekennzeichneten Mitosen der extrakapsulären Körper wurden von BĚLAŘ (1926) genauer analysiert. Er konnte das Auftreten des Längspaltes in den Prophasechromosomen und das Auseinanderweichen der Chromatiden verfolgen. Ich fand seine klaren Bilder, abgesehen von BĚLAŘs zu niedriger Schätzung der Chromosomenzahl und der angeblichen Auflösung des Nukleolus, in allen wesentlichen Punkten

bestätigt und darf mich daher im folgenden des öfteren auf seine Fig. 88 beziehen.

Meine Befunde stellen insofern eine Fortsetzung der BĚLAŘschen Untersuchung dar, als jene den von BĚLAŘ in so ausgedehntem Masse geführten Nachweis der Wesensgleichheit karyologischer Grundphänomene bei Protisten und höheren Organismen auf zwei Prinzipien ausdehnen, die erst in den letzten Jahren bei Metazoen und Metaphyten entdeckt oder doch wenigstens in ihrer allgemeinen Bedeutung gewürdigt wurden, nämlich 1. die von HEITZ als gesetzmäßig erkannte Lage der Nukleolen an einer achromatischen Einschnürung sog. SAT-Chromosomen und 2. die Spiralstruktur der Chromosomen. Kürzlich hat GEITLER (1936) erstmalig auch an Protisten (Cladophoraceen) Spiralstruktur der Chromosomen nachgewiesen und die Gültigkeit des 1. Prinzips sehr wahrscheinlich gemacht. Der Grad der Allgemeingültigkeit scheint allerdings verschieden zu sein. Denn während kein Anlaß vorliegt, zu bezweifeln, daß allgemein, wenn auch nicht ausschließlich, der Formwechsel der Chromosomen auf wechselnder Ausbildung von Spiralen beruht, gilt die Beziehung Nukleolus-SAT-Chromosom bei Protisten sicher nicht in allen Fällen. Hierauf weist GEITLER hin und bemerkt, daß die Regelung durch SAT-Chromosomen als Ausdruck einer höheren Differenzierung aufgefaßt werden kann. Ich gehe auf die Nukleolenfrage und das Schrifttum dazu nicht weiter ein, sondern verweise auf das Sammelreferat von HEITZ (1935).

Spiralbau der Chromosomen (Literaturangaben findet man in den Arbeiten von HEITZ und GEITLER 1935) hat sich in einer so großen Zahl von Fällen demonstrieren lassen, daß die Verallgemeinerung berechtigt ist, am Formwechsel aller Chromosomen sei die wechselnde Bildung und Auflösung von Spiralen wesentlich beteiligt. Daß der Nachweis einer anzunehmenden Spiralstruktur in gewissen Stadien (minor coils in Pro-, Meta- und Anaphase) gar nicht oder nur an günstigen Objekten gelingen will, erklärt sich aus der häufig offenbar submikroskopischen Größe der Windungen, aus dem zeitweiligen Vorhandensein einer das spiralisierte Chromonema verdeckenden Matrix (HEITZ schlägt vor, diesen etwas unglücklichen Terminus durch das Wort Kalymma—Hülle—zu ersetzen) und schließlich aus der Fixierungslabilität so zarter Strukturen. Bei somatischen Chromosomen konnte bisher nur eine Spirale (minor coils) nachgewiesen werden<sup>1)</sup>, und die Untersuchungen im polari-

1) HEITZ (1935) erweckt den gegenteiligen Anschein, wenn er schreibt, nach neueren Untersuchungen sei „jedes Spiral-Chromonema nochmals in sich spiralig gewunden“, doch berichten die von ihm in diesem Zusammenhang angeführten Arbeiten von doppelter Spiralisierung nur aus der Meiose.

sierten Licht von KUWADA und NAKAMURA (1934) können mit Vorbehalt dahin gedeutet werden, daß wenigstens in vielen Fällen auch nur eine Spirale vorhanden ist. Von den am ehesten klar darstellbaren Spiralen der Chromosomen aus der ersten meiotischen Teilung hat sich dagegen in einer Reihe von Fällen (DARLINGTON, FUJII, KATO u. IWATA, KUWADA u. NAKAMURA u.a.) zeigen lassen, daß es sich um major coils handelt, die den hier fast oder ganz submikroskopischen minor coils überlagert sind. Bei alledem ist vor unberechtigten Verallgemeinerungen zu warnen. Es ist sehr wohl denkbar, daß in somatischen Chromosomen das Chromonema in der Regel nur zu minor coils, in anderen Fällen aber außerdem noch zu zusätzlichen Spiralen höherer (major coils) oder niederer Ordnung (minimum spiral, DARLINGTON) aufgewunden ist. Daß bei *Drosophila* die Metaphasechromosomen nur etwa ein Hundertstel der Länge des in Speicheldrüsenkernen meßbaren Chromonemas aufweisen, spricht unter der Voraussetzung einer einigermaßen konstanten Chromonemalänge durchaus für eine „spiral within spiral“-Struktur. Andererseits deuten Fälle, in denen somatische Chromosomen dieselbe Länge aufweisen wie die Chromatiden der ersten meiotischen Metaphase, darauf hin, daß hier somatische wie meiotische Chromosomen major und minor coils oder beide nur minor coils besitzen.<sup>1)</sup>

Bei den „Spiralen“ der Chromosomen handelt es sich natürlich niemals um Spiralen im mathematischen Sinne, sondern um Schraubenlinien, die höchstens streckenweise (etwa in der Nähe des Spindelansatzes und an den Enden des Chromosoms) in Turbospiralen übergehen. Da also keine Verwechslungsgefahr besteht, und die Bezeichnung sich eingebürgert hat, behalte ich sie hier bei. Eine Schraube wird, abgesehen vom Windungssinn, eindeutig charakterisiert durch den Radius des von ihr umhüllten Zylinders und durch das Verhältnis der Länge der Schraubenlinie zur Länge des Zylinders.

1) Einen solchen Fall fand ich bei *Aranea reaumuri* SCOP., worauf ich an anderer Stelle zurückkommen werde. Im Ovar gibt es zwei Typen von Mitosen mit auffallend verschiedener Gesamtchromosomenlänge (ob beide oder welcher von ihnen Oogonienteilungen darstellt, kann ich nicht mit Sicherheit sagen; die Dicke der Metaphasechromosomen ist bei dem kurzarmigen Typ wesentlich größer). Ferner stellte ich die ungefähre Gesamtchromosomenlänge der ersten Oocytenteilung und die des Pachynemas fest. Die haploide Chromosomenlänge des einen Mitosetypus verhält sich zu der des Pachynemas etwa wie 1:5,6, die entsprechenden Proportionen für den anderen Typus und für die erste Oocytenteilung betragen etwa 1:9,8 und 1:9,2. Ob der Unterschied zwischen den letzten beiden Zahlen real ist, kann ich nicht sagen, auch weiß ich nicht, ob die Pachytänchromosomen gegenüber dem Leptonema bereits verkürzt sind. Das Ergebnis ist mit beiden oben erwähnten Möglichkeiten verträglich, scheint mir aber mehr dafür zu sprechen, daß den anzunehmenden minor coils des 1. Mitosetypus im 2. Fall und in der Meiose noch major coils überlagert sind.

In diesem Sinne spreche ich vom Radius, der die Dicke des Chromosoms und vom Verkürzungsfaktor, der seine Länge bestimmt.

Die für den Formwechsel eines somatischen Chromosoms während einer mitotischen Periode wesentlichsten Veränderungen im Spiralbau sind offenbar folgende (eine eingehendere Interpretation der Mitose gibt DARLINGTON 1935): während der Prophase legen sich in jeder Chromatide<sup>1)</sup> neue Windungen an, deren zunächst sehr kleiner Radius und deren Verkürzungsfaktor allmählich zunehmen. Das Chromosom wird also dicker und kürzer. Während der Meta- und Anaphase befindet sich das Chromosom im allgemeinen in einem stabilen Zustand. In der Telophase wird der Radius wieder größer, und die Windungen lockern sich auf, werden zu Reliktwindungen (relic coils, DARLINGTON). Diese Bewegung wird im Ruhekern, dem zweiten mechanisch stabilen Zustand, eingestellt. Das im Ruhekern höchstens andeutungsweise verfolgbare Chromonema tritt in der frühen Prophase (spiral stage) in ähnlichen Reliktwindungen auf, wie sie der Ruhekern aus der späten Telophase übernommen hatte. Der Prozeß der Auflockerung schreitet nun weiter fort und zwar für die jetzt oder früher in Erscheinung tretenden Chromatiden eines Chromosoms gemeinsam. Noch vor dem völligen Verschwinden der Reliktspiralen beginnt ein neuer Zyklus durch Anlage der sich in jeder Chromatide gesondert entwickelnden neuen Spiralen.

Dieses Bild vom Ablauf der mitotischen Periode wird von der Metaphase bis zur Auflösung der Reliktspiralen in der Prophase durch zahlreiche Beobachtungen verschiedener Autoren gestützt. Die Erklärung der prophasischen Verkürzung und Verdickung der Chromosomen durch Anlage neuer Spiralen erscheint als selbstverständliche Ergänzung, da die nicht gut zu bezweifelnde Rolle des Chromonemas als Träger der Genkette die früher mögliche Vorstellung, das in der Metaphase sichtbar werdende spiralisierte Chromonema werde jedesmal neugebildet, heute unhaltbar macht. Die Entwicklung der Reliktspiralen von der Telophase bis zur Prophase hat bereits BONNEVIE (1908) bei *Ascaris* und *Allium* gesehen und im modernen Sinne gedeutet; schon aus ihren Ergebnissen ging klar hervor, daß, worauf neuerdings DARLINGTON aufmerksam macht, die Telophase nicht als Umkehrung der Prophase bezeichnet werden darf. Vollkommen offen bleibt einstweilen die Frage, ob das Chromonema eine im wesentlichen konstante Länge besitzt oder merklicher Kontraktion fähig ist. Als Arbeitshypothese verdient die einfachere erste Annahme den Vorzug.

1) Die sehr umstrittene Frage nach dem Zeitpunkt der Chromosomenverdoppelung kann hier unerörtert bleiben.

## II. Objekt und Technik

Die aus dem Golf von Neapel stammenden *Collozoum*-Kolonien wurden grösstenteils zu Karminessigpräparaten verarbeitet. Die Färbung wurde entweder durch Eindunstenlassen auf dem Objektträger oder durch vorsichtiges Erwärmen unter dem Deckglas verstärkt. Überführung in Kanadabalsam erfolgte nach dem von BUCK (1935) angegebenen Verfahren über Eisessig-Alkohol-Xylol, Alkohol-Xylol. Zur Kontrolle wurden Ausstriche über Osmiumsäure geräuchert, mit BOVIN-ALLEN nachfixiert und nach HEIDENHAIN gefärbt, ferner in BOVIN-ALLEN fixierte Kolonien geschnitten und teils nach FEULGEN, teils nach HEIDENHAIN gefärbt. Den Zeichnungen liegen, sofern nicht anders bemerkt, Karminessigpräparate zugrunde.

## III. Der Chromosomensatz

Von der späten Prophase bis zum Ende der Anaphase wird eine genauere Analyse der Chromosomen durch ihre dichte Lagerung unmöglich gemacht (vgl. BĚLAŘS Fig. 88). Erst das ungemein charakteristische mittlere Telophasestadium der Fig. 1a läßt eine Zählung zu, die aber auch hier durch die stets bestehende Zusammendrängung der Spindelansatzpunkte und die lockere



Fig. 1. a-b Telophase; in a sind die aus der Ebene herausragenden Chromosomen punktiert; b das SAT-Chromosom (im optischen Schnitt). Vergr. etwa 2500 $\times$ . c Schraube mit dem Verkürzungsfaktor 5,5.

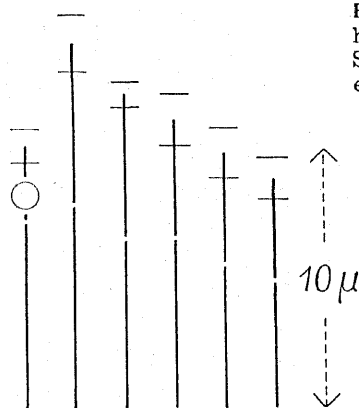


Fig. 2. Die Längen der Chromosomen und die Lage ihrer Spindelansatzpunkte nach Messungen an 5 Telophasekernen; die horizontalen Striche bezeichnen die mittleren Abweichungen.

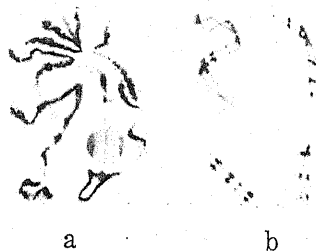


Fig. 3. a der Pol des in Fig. 6 vollständig gezeichneten mittleren Prophasekernes. b Chromatiden mit Chromomeren, teils aus dem Kern der Fig. 6, teils aus anderen Kernen desselben Stadiums. Vergr. etwa 2500 $\times$ .

Struktur der Chromosomen erschwert wird. Häufig liegen 8 freie Enden ungefähr in einer Ebene, während 4 weitere leicht übersehbar aus ihr herausragen. So erklärt sich BĚLAŘS Bemerkung, es handle sich wahrscheinlich um 4 bügelförmige Chromosomen<sup>1)</sup>. Es sind 6 Chromosomen mit nahezu medianem Spindelansatz. 12 distale Enden können fast immer sicher ausgemacht werden; dagegen sind die proximalen Teile oft unklar, doch lassen die Fälle, in denen 1 Chromosom etwas isoliert liegt und dann stets deutlich V-förmig ist, sowie Prophasen, in denen die Spindelansatzregionen manchmal sehr klar nebeneinander liegen (Fig. 3a), an der zweiarmigen Gestalt sämtlicher Chromosomen keinen Zweifel. Ein Chromosom ist als Nukleolen- oder SAT-Chromosom ausgebildet.

5 Telophasekerne der zweiten Teilung, deren Spindelansatzregionen für genügend klar befunden wurden, um jedem Chromosomenarm mit ausreichender Sicherheit den anderen Arm zuzuordnen zu können, wurden ausgemessen und durch Berücksichtigung von Höhendifferenzen die wahre Länge aller Chromosomen ermittelt. Die Gesamtlänge beträgt  $65 \pm 1\mu$ . Obwohl die Meßgenauigkeit unter der unscharfen Kontur der aufgelockerten Chromosomen litt, ist der Fehler des Mittelwertes auffällig klein. Das verstärkt den Eindruck, den man bereits aus der großen Häufigkeit dieses Telophasestadiums gewinnt, daß hier ein stabiler Zustand vorliegt. Es ist auch der einzige während der Mitose, denn von der Prophase bis zur Telophase scheint die Verkürzung und vor allem die Verdickung der Chromosomen kontinuierlich fortzuschreiten (vgl. BĚLAŘS Fig. 88)—jedenfalls tritt keines der durchlaufenen Stadien mit einer Häufigkeit auf, die an die der mittleren Telophase heranreicht. Der stabile Zustand, der bei den meisten Organismen mit der Meta- und Anaphase zusammenfällt, stellt sich hier also erst in der Telophase ein.

Fig. 2 gibt das Ergebnis der Längenmessung an den einzelnen Chromosomen wieder, die in jedem der 5 Kerne nach der relativen Länge seriiert wurden. Es spricht durchaus dafür, daß alle Chromosomen verschiedene Länge und mehr oder weniger verschiedene Lage des Spindelansatzes aufweisen, wiewohl dieser Schluß in Anbetracht des nicht genügend kleinen mittleren Fehlers nicht als völlig gesichert gelten kann. Das deutet aber darauf hin, daß es sich um einen haploiden Satz handelt.

1) TROJAN (1930) will 5 Chromosomen gezählt haben. Die Behauptung wird durch die merkwürdigen Abb. 7-8 kaum ausreichend belegt, deren Veröffentlichung 4 Jahre nach dem Erscheinen der—von TROJAN zitierten!—BĚLAŘSchen Arbeit schwer zu verstehen ist.

#### IV. Das SAT-Chromosom

Der Nukleolus umschließt stets eine achromatische Fibrille, die den Satelliten mit dem übrigen Chromosom verbindet (Fig. 1 a-b, 3a, 4d); auf die Fibrille folgt proximal regelmäßig ein Chromomer, dann eine achromatische Stelle, dann wieder eine färbare Region, die in den anderen Chromosomenarm überleitet und in der offenbar der Spindelansatz zu suchen ist, wie Fig. 3a und 4d zeigen, in denen an dieser Stelle das Chromonema scharf umknickt. Eine besondere Differenzierung der Spindelinsertion konnte ich nicht nachweisen. Der Nukleolus besteht aus einer inneren, mehr oder weniger kugelförmigen Substanz und einer äußeren, deren Anordnung wechselt. Beide verhalten sich wie die Fibrille, die jene innere Schicht median durchsetzt, FEULGEN-negativ und sind dementsprechend auch mit Karminessigsäure kaum färbbar; erst bei sehr kräftiger Behandlung erfolgt eine merkliche Anfärbung der äußeren Schicht. Diese allein färbt sich, und zwar sehr intensiv, nach HEIDENHAIN, scheint also dichter zu sein (Fig. 4a). Ob die äußere Substanz der inneren nur teilweise aufgelagert ist, oder sie in sehr dünner Schicht auch an den anderen Stellen umgibt, konnte ich nicht entscheiden.

Die Fibrille ist im Nukleolus in genügend gefärbten Präparaten stets erkennbar, im allgemeinen auch ihr beiderseitiger Anschluß an färbbare Regionen des Chromonemas. Sie kann, sei es bereits im Leben (dafür spricht Fig. 4d), sei es im Gefolge artifizierlicher Verlagerungen, passiv stark gedehnt oder gar zerrissen werden.

Als artifizell dürfte die häufig isolierte Lage des Nukleolus in der frühen Prophase (Fig. 5c) zu deuten sein. Mittlere Prophasen, in denen auch an anderen Chromosomenstellen der Längsspalt sichtbar wird, lassen gelegentlich zwei Fibrillen im Nukleolus erkennen, doch sind klare Bilder selten (Fig. 4b-c).

In der Prophase bildet die dichtere Substanz des Nukleolus einen mehr oder weniger gleichmäßigen Wulst um dessen inneren Teil (Fig. 3a, 5c). Von der späten Prophase bis zur Trennung der Tochter-satelliten in der mittleren Anaphase entzieht sich der Nukleolus meist einer genauen Untersuchung, da er durch seine Lage am SAT-

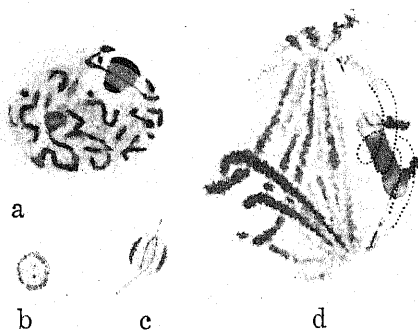


Fig. 4. a Früher Prophasekern, nach HEIDENHAIN gefärbt. b-c Nukleoli mit geteilter Fibrille aus mittleren Prophasekernen (a-c im optischen Schnitt). d Anaphase (die langen Arme der SAT-Chromosomen punktiert). Vergr. etwa 2500 $\times$ .



Chromosom in der Nähe des Kernpoles festgehalten und durch die hier mit ihren Spindelansätzen zusammengedrängten, ständig dicker werdenden Chromosomen leicht verdeckt wird. Schwache Färbung läßt am Nukleolus nichts erkennen, starke Färbung macht die Chromosomenmasse recht undurchsichtig. Es kann also nicht Wunder nehmen, daß ich in einigen wenigen Fällen den Nukleolus nicht sicher nachweisen konnte, obwohl ich es für unzweifelhaft halte, daß er nicht aufgelöst wird, sondern die Mitose im wesentlichen unverändert überdauert. Dafür scheint mir die in Fig. 4d wiedergegebene Anaphase beweisend zu sein, die schwerlich als Ausnahme betrachtet werden kann, da ich genau dasselbe Bild noch ein zweites Mal gefunden habe, ferner ein etwas späteres Stadium mit anscheinend kurz vor der Trennung stehenden Tochternukleolen. Offenbar wird erst die innere Substanz durchgeschnürt, dann die zähere äußere. Einen Hinweis darauf, daß dies der normale Vorgang ist, sehe ich auch in der stets zu beobachtenden exzentrischen Lage der äußeren Nukleolensubstanz in der Telophase (Fig. 1a-b). Die Möglichkeit, daß der Nukleolus ganz oder teilweise aufgelöst und noch vor der Trennung der Tochttersatelliten neugebildet wird, kann als durchaus unwahrscheinlich wohl beiseite gelassen werden.

Die Angaben von HARTMANN und HAMMER und von BĚLAŘ, wonach der Nukleolus aufgelöst wird, sind vermutlich auf den Umstand zurückzuführen, daß der Nukleolus hier gerade in den Stadien am schwersten aufzufinden ist, in denen er sonst aufgelöst wird und in denen man daher auch hier zunächst eine Auflösung erwartet. Die Fibrille im Nukleolus wurde übrigens schon von den genannten Autoren beobachtet, aber verständlicherweise falsch gedeutet, da die Nukleolus-SAT-Beziehung damals noch unbekannt war. Die hier gefundene Persistenz der Nukleolen ist nicht der einzige Fall dieser Art. GEITLER (1936) fand bei verschiedenen Cladophoraceen alle Übergänge zwischen „weitgehender Persistenz der Nukleolen und Neubildung in den Tochterkernen.“

## V. Die Spiralstruktur der Chromosomen

Die Telophasechromosomen zeigen unzweifelhaften Spiralbau, worauf bereits BĚLAŘS Fig. 88g hindeutet. Die Windungen lassen sich allerdings nur gelegentlich und dann in der Nähe des Spindelansatzes verfolgen (Fig. 1a-b), in einiger Entfernung von ihnen bekommt man in der Regel höchstens für ganz kurze Strecken klare Bilder. Die Spiralstruktur als einzig mögliche Deutung wird freilich dadurch nicht in Frage gestellt. Wohl aber erhebt sich das Problem, ob das spiralisierte Chromonema bereits geteilt ist. Manchmal glaubt man, streckenweise, jedoch nie in der Nähe des Spindelansatzes,

Andeutungen davon zu bemerken, die aber auch anders ausgelegt werden können. Direkte Beobachtung führt in meinen Präparaten nicht weiter und an sich ist beides denkbar. Der Längsspalt kann erst in der mittleren Prophase mit Sicherheit festgestellt werden, wann er in Wirklichkeit auftritt, bleibe dahingestellt.

Die Schwierigkeit, den Windungen des Chromonemas genauer zu folgen, erklärt sich außer durch artifizielle Veränderungen schon durch dessen Aufbau aus Chromomeren mit dazwischengeschalteter Fibrille, wie wir ihn oben am SAT-Chromosom zwischen Spindelansatz und nukleolustragender Fibrille kennenlernten (die selber wenigstens morphologisch nichts anderes ist als ein besonders langes chromomerenloses Stück des Chromonemas), und wie er natürlich auch in den anderen Abschnitten anzunehmen ist. Chromosomen der mittleren Prophase zeigen manchmal diese Struktur des Chromonemas recht deutlich (s. unten). Möglicherweise sind die an sich achromatischen interchromomeralen Fibrillen in verschiedenen Stadien in verschiedenem Maße mit färbbarer Substanz beladen, und auch die Größe und Färbbarkeit der Chromomeren braucht nicht als konstant angenommen zu werden. Schließlich wird die Analyse der Telophasespiralen durch ihren unregelmäßigen Verlauf erschwert, der vielleicht auf das Fehlen (wenigstens im fixierten Präparat) einer nachweisbaren Matrix zurückzuführen ist.

In der späten Telophase drängen sich die Chromosomen zusammen und werden dann schnell in einen verhältnismäßig kleinen Ruhekern eingeschlossen (Fig. 5a-b). Hier kann

das Chromonema, außer gelegentlich über kürzeste Strecken, nicht mehr verfolgt werden, wofür wohl nur z.T. die enge Lagerung verantwortlich zu machen ist; anscheinend sind in diesem Stadium nur noch die Chromomeren färbbar, so daß der Kern ein granuläres Aussehen annimmt. Immerhin lassen undeutlich schattenhafte Umrisse die im

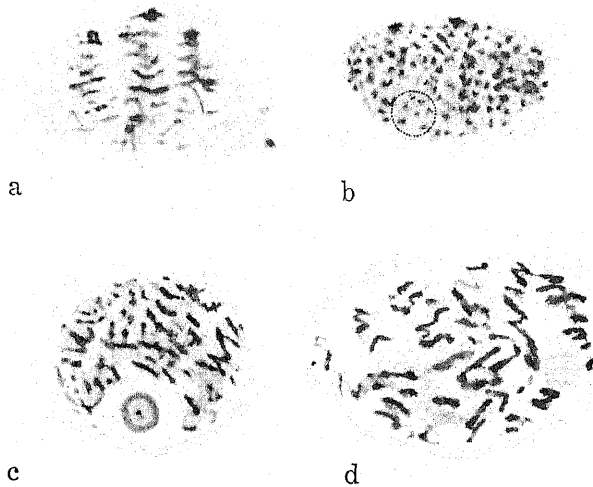


Fig. 5. a Späte Telophase. b Ruhekern (der in der Tiefe liegende Nukleolus punktiert). c-d Frühe Prophase. Optische Querschnitte. Vergr. etwa 2500 $\times$ .

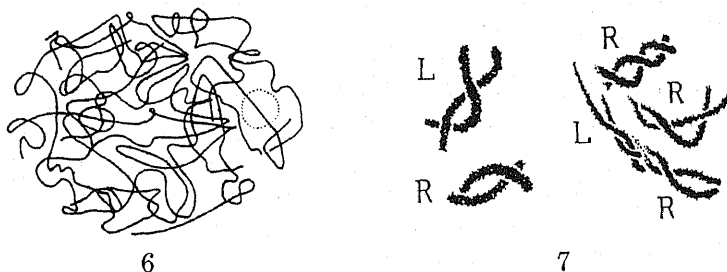
wesentlichen parallele Orientierung der Chromosomen erkennen, der sich auch die Lage des Nukleolus und der gleich zu besprechenden Chromozentren einfügt.

Eine Besonderheit der Chromosomenenden, zu denen auch der Satellit rechnet, muß nachgetragen werden. In der Telophase sind diese stärker als die übrigen Teile gefärbt (Fig. 1a-b). Das beruht anscheinend darauf, daß die distalen Windungen des Chromonemas noch den kleineren Radius früherer Stadien beibehalten haben. Dieser Zustand erhält sich bis in den Ruhekern, in dem daher die Chromosomenenden als Chromozentren sichtbar bleiben. Die Erscheinung ist nicht ausgeprägt genug, um eine sichere Zählung zu erlauben und verschwindet in späteren Stadien überhaupt. Da kein Anlaß vorliegt, hinter dieser Erscheinung mehr zu sehen als eine durch die besonderen mechanischen Bedingungen der Chromosomenenden verursachte Verzögerung in der telophasischen Auflockerung der Metaphasespiralen, darf diese „Pseudoheterochromatie“ nicht mit der „echten“ Heterochromatie zusammengeworfen werden, die vor allem von HEITZ an den verschiedensten Objekten studiert wurde. BAUER (1936 a, b) hat gezeigt, daß diese wenigstens bei den untersuchten Dipteren (Chironomiden, *Drosophila*) mit einem abweichenden Bau sog. Heterochromomeren zusammenhängt. Wie MULLER und PROKOFYEVA (1935) bemerkten, weisen bei *Drosophila* die Längenverhältnisse der heterochromatischen und euchromatischen Abschnitte von Speicheldrüsenchromosomen und normalen Chromosomen darauf hin, daß in diesen die heterochromatischen Abschnitte nicht (oder weniger) spiralisiert sind als die euchromatischen. Das könnte rein mechanisch durch die bedeutendere Größe der Heterochromomeren bedingt sein. Von geringerer Spiralisierung der „heterochromatischen“ Abschnitte ist, wie schon der Vergleich der Satellitenlänge in Telo- und Prophase lehrt (Fig. 1a-b, 3a), bei unserem Objekt sicher nicht die Rede und für das Vorhandensein besonderer Heterochromomeren habe ich kein Anzeichen finden können.

In den untersuchten extrakapsulären Körpern folgt offenbar Mitose auf Mitose, und das Ruhestadium besteht nur kurze Zeit. Während der Prophase wächst der Kern beträchtlich (vgl. Fig. 5b-d, 6), die Färbbarkeit des Chromonemas nimmt zu, und Reliktspiralen werden sichtbar. Fig. 5d ist ein typisches Bild eines häufig zu findenden Stadiums. Bei der weiteren Auflösung der Reliktspiralen strecken sich die Chromosomen<sup>1)</sup> immer mehr und verlieren infolge-

1) Unter Chromosomen sei hier, wie schon in der Telophase, der von der Chromonemaspirale umschlossene Raum verstanden. Er scheint sich in nichts von der übrigen Karyolymphe zu unterscheiden, gegen die offenbar keine Abgrenzung besteht. Es ist eine rein geometrische Definition, die nur in den Stadien, in denen eine Matrix ausgebildet wird, materiellen Inhalt erhält; ob das bei unserem Objekt überhaupt eintritt, kann ich nicht sagen. Das alte Chromosom verschwindet mit der Auflösung der Reliktspirale, erhalten bleibt nur das Chromonema.

dessen die in der späten Telophase und im Ruhekern so auffällige parallele Orientierung, da der beschränkte Kernraum sie zwingt, sich in unregelmäßiger Weise aufzuknäulen (Fig. 6). In dieser mittleren Prophase wird der Spalt erkennbar und die allmähliche Verkürzung und Verdickung der umeinandergewundenen Chromatiden setzt ein. Dieses Umeinandergewundensein (relational coiling, DARLINGTON) der Chromatiden spielt in den Erörterungen über den Mechanismus des Crossing-over eine bedeutende Rolle. Da in diesen Stadien eine Untersuchung aller Chromosomen eines Kernes oder gar die Identifizierung bestimmter Chromosomen (selbst bei dem langen Arm des



Figs. 6-7. 6. Mittlerer Prophasekern der 3. Teilung. Vergr. etwa 2500 $\times$ . 7. Relational coiling aus zwei Anaphasen (die Chromosomen sind nicht in voller Länge dargestellt). Vergr. etwa 2500 $\times$ .

SAT-Chromosoms war das kaum jemals möglich) ausgeschlossen schien, hatte eine nähere Analyse wenig Zweck. Rechts- und Linkswindungen treten nebeneinander (Fig. 7) und anscheinend mit gleicher Häufigkeit auf: von 19 Chromatidenpaaren aus verschiedenen Kernen waren 10 rechts- und 9 linksgewunden. Windungsumkehr innerhalb eines Armes wurde nicht beobachtet. Zusammenfassend kann gesagt werden, daß die Mitose, abgesehen von der abweichenden Lage des einen der beiden stabilen Zustände, den von höheren Organismen bekannten und in der Einleitung skizzierten Verlauf nimmt.

In Kernen der mittleren Prophase mit bereits erkennbarem Längsspalt treten manchmal auf den nebeneinanderliegenden Chromatiden (die sich oft überkreuzen, also anscheinend bereits hier umeinandergewunden sind) Strukturen auf, die, wenn klare Bilder auch nicht häufig sind, doch wohl unbedenklich als Chromomeren mit verbindender achromatischer Fibrille gedeutet werden dürfen (Fig. 3b). Wichtig ist nun, daß diese Bilder auf einen (von gröberen Windungen abgesehen) im wesentlichen gestreckten Zustand des Chromonemas schließen lassen. Das eröffnet die Möglichkeit, die Gesamtlänge des Chromonemas zu ermitteln, um sie mit der Länge der Telophasechromosomen und mit deren Spiralbau in Beziehung zu setzen.

Natürlich kann die Länge der aufgeknäulten Chromonemata nicht direkt festgestellt werden, doch läßt sich in günstigen Fällen wenigstens die Länge ihrer Projektion messen. Der in Fig. 6 wiedergegebene Kern erwies sich als geeignet. Mit dem ABBÉschen Apparat wurden sämtliche Windungen gezeichnet und mit dem Kurvenmesser die Länge entnommen. Der erhaltene Wert ist sicher recht genau, obwohl der Kern an manchen Stellen unklar ist, denn für die Länge ist es ohne Belang, wie man etwa die in einen Knoten einmündenden Fäden miteinander verknüpft.

Aus der Länge der Projektion ( $l_p$ ) einer Raumkurve kann aber unter einer gleich zu erleuternden Voraussetzung, die ich die „Voraussetzung ungeordneter Orientierung“ nennen will, ihre wahre Länge  $l$  in einfachster Weise berechnet werden. Mit jener Voraussetzung soll folgendes gefordert werden: von den Tangenten der Kurve werde keine Richtung bevorzugt. Schärfer formuliert: die (rektifizierbare) Kurve werde in  $n$  Abschnitte gleicher Sehnenlänge zerlegt. Die Sehnen werden unter Beibehaltung ihrer Orientierung so an einen Punkt angetragen, daß sie sämtlich auf dieselbe Seite einer durch den Punkt gehenden Ebene oder, so weit sie in die Ebene fallen, auf dieselbe Seite einer durch den Punkt gehenden Gerade dieser Ebene zu liegen kommen. Die Voraussetzung besteht dann darin, daß mit zunehmender Feinheit der Unterteilung die Verteilung der Sehnenenden auf der von ihnen bestimmten Kugelfläche gegen vollkommene Gleichmäßigkeit konvergiert (für die Rechnung muß dieser Satz natürlich noch schärfer gefaßt werden). Dann ergibt sich

$$\frac{l_p}{l} = \lim_{n \rightarrow \infty} \frac{\sum \cos \varphi}{n} = \int_0^{\pi/2} \cos^2 \varphi d\varphi$$

dabei bedeutet  $\varphi$  den Winkel einer Sehne gegen die Ebene. Es ist also

$$l = \frac{4}{\pi} \cdot l_p.$$

Die Forderung ungeordneter Orientierung ist in dem Kern der Fig. 6 offenbar annähernd erfüllt. Für die Gesamtlänge ergibt sich etwa  $330 \mu$ . In einem anderen Kern ermittelte ich  $415 \mu$ , doch kann dieser Wert als nicht so sicher gelten wie der erste, da der Verlauf der Chromosomen hier unsicherer ist und vor allem, da es sich um einen augenscheinlich stark gepreßten Kern handelt, bei dem die in der Zeichnung vorliegende Projektion der wahren Länge wahrscheinlich näher kommt, der Faktor  $\frac{4}{\pi}$  also zu groß ist. Dennoch scheint mir ein realer Unterschied zu bestehen, der darin begründet sein mag, daß im zweiten Kern die Chromonemata noch vollständiger ausge-

glättet sind als im ersten. Der wahre Wert, dessen genaue Ermittlung hier ohne Interesse ist, da die Telophasespiralen doch nur einen ungefähren Vergleich zulassen, könnte also zwischen den angegebenen liegen. Nehmen wir an, er betrage  $360 \mu$ . Der Vergleich mit der oben mitgeteilten Gesamtlänge der Telophasechromosomen liefert einen Verkürzungsfaktor von etwa 5,5. In Fig. 1c habe ich eine Schraube gezeichnet, die diesen Verkürzungsfaktor und etwa den Radius der Telophasechromosomen aufweist. Es ist deutlich zu sehen, daß die Windungen der errechneten Schraube von derselben Größenordnung sind wie die der Telophasechromosomen (Fig. 1a-b), mögen deren Windungen auch unregelmäßig und im Einzelnen unklar sein. Da der mikroskopische Befund keinen Anhalt dafür gibt, daß der spiralisierte Faden etwa seinerseits noch eine feine Spirale darstellt, entspricht das Ergebnis der in der Einleitung vertretenen Arbeitshypothese, nach der das Chromonema seine Länge in allen Stadien im Wesentlichen bewahrt.

## VI. Zusammenfassung

Von den 6 V-förmigen Chromosomen ist eines das SAT-Chromosom; die anderen unterscheiden sich in der Größe voneinander, anscheinend handelt es sich um den haploiden Satz.

Der Nukleolus umgibt den als achromatische Fibrille ausgebildeten Teil des Chromonemas, der den Satelliten mit dem übrigen Chromosom verbindet. Am Nukleolus sind ein innerer Teil, der von der stets sichtbar bleibenden Fibrille median durchsetzt wird und sich nach HEIDENHAIN nicht färbt und ein stark färbbarer äußerer zu unterscheiden. Der Nukleolus wird während der Mitose nicht aufgelöst.

Die Telophasechromosomen lassen deutlichen Spiralbau erkennen. In der Prophase kann die Auflockerung der Reliktspiralen verfolgt werden. Die Mitose unterscheidet sich von dem bei höheren Organismen bekannten Verlauf nur darin, daß hier die Verkürzung und vor allem die Verdickung der Chromosomen bis zur mittleren Telophase anhält, diese erst darf als stabiler Zustand der Chromosomen gelten.

Die Chromosomenenden zeigen „Pseudoheterochromatie“, die offenbar auf verzögerter Auflockerung beruht und anscheinend nur bis in den Ruhekern hinein besteht.

Es wird ein Verfahren angegeben, mit dessen Hilfe unter der „Voraussetzung ungeordneter Orientierung“ die Gesamtlänge eines Spirems ermittelt werden kann.

Die Gesamtlänge der Telophasechromosomen wird mit der des offenbar weitgehend ausgeglätteten Chromonemas der mittleren Pro-

phase verglichen und als Verkürzungsfaktor 5,5 festgestellt. Eine diesem Faktor entsprechend gezeichnete Schraube sieht der in der Telophase erkennbaren ähnlich. Das spricht zugunsten der Arbeitshypothese, daß das Chromonema in allen Stadien etwa dieselbe Länge habe.

#### VII. Schriftenverzeichnis

- Bauer, H., 1936a. Beiträge zur vergleichenden Morphologie der Speicheldrüsenchromosomen. Zool. Jahrb. Phys. 56, 239-276.
- — 1936b. Structure and arrangement of salivary gland chromosomes in *Drosophila* species. Proc. nat. Acad. Sci. U.S.A. 22, 216-222.
- Bělař, K., 1926. Der Formwechsel der Protistenkerne. Ergebnisse u. Fortschr. d. Zool. 6, 135-154.
- Bonnevie, K., 1908. Chromosomenstudien. Arch. Zellforschung 1, 450-514.
- Buck, J. B., 1935. Permanent aceto-carmin preparations. Science 81, 75.
- Chatton, E., 1923. Les péridiniens parasites des radiolaires. C. r. Acad. Sci. Paris 177, 1246-1248.
- — 1934. L'origine péridinienne des radiolaires et l'interprétation parasitaire de l'anisoprogénèse. C. r. Acad. Sci. Paris 198, 309-312.
- Darlington, C. D., 1935. The internal mechanics of the chromosomes. I. The nuclear cycle in *Fritillaria*. Proc. Royal Soc. London. B. 118, 33-59.
- Geitler, L., 1935. Neueste Forschungen über den Chromosomenbau. Züchter 7, 294-304.
- — 1936. Vergleichende Untersuchungen über den feineren Kern- und Chromosomenbau der Cladophoraceen. Planta 25, 530-578.
- Hartmann, M. u. E. Hammer, 1909. Untersuchungen über die Fortpflanzung von Radiolarien. Sitzber. Ges. naturf. Freunde Berlin 228-248.
- Heitz, E., 1935. Chromosomenstruktur und Gene. Ztschr. ind. Abst. 70, 402-447.
- Kuwada, Y. and T. Nakamura, 1934. Behaviour of chromonemata in mitosis, IV. Double refraction of chromosomes in *Tradescantia reflexa*. Cytologia 6, 78-86.
- Muller, H. J. and A. A. Prokofyeva, 1935. The structure of the chromonema of the inert region of the X-chromosome of *Drosophila*. C. r. d. Acad. d. Sci. 1, 658-660.
- Trojan, E., 1930. Zur Streitfrage der systematischen Stellung der Radiolarien. Zool. Anzeiger 90, 146-155.

## Karyotypic Polymorphism in *Paris hexaphylla* Cham., with Special Reference to Its Origin and to the Meiotic Chromosome Behavior

By

Tutomu Haga

Botanical Institute, Faculty of Science,  
Hokkaido Imperial University, Sapporo

Since its first discovery by NAWASCHIN (1912) in *Galtonia candicans*, karyotypic polymorphism has been fully identified in many other plants (cf. KUHN 1928 and ONO 1935). Interestingly recent investigations dealing with the karyotypic aberration have furnished, in certain cases, karyological bases for such genetic phenomena as balanced lethal mechanism (MALINOWSKI 1935), partial sterility (NAVASHIN 1933), vigorousty (MEDWEDWA 1929) etc. On the contrary in certain cases it is inferred that the polymorphism is concerned only with the genetically inert portion of the chromosome (LESLEY and LESLEY 1935).

The polyploids and karyotypes in *Paris hexaphylla* CHAM.<sup>1)</sup> were found by GOTOH and STOW (1930),<sup>2)</sup> and preliminarily reported by the present writer (1934 a, b and 1935). Studying the same plant the writer confirmed the observations by the former investigators, revealing six karyotypes of which four are diploid ( $2n = 10$ ) and two triploid ( $2n = 15$ ).

In this paper the writer gives a detailed description of the somatic complements of six karyotypes with some features of the meiotic chromosome behavior.

### Material and Methods

The material used was collected at the foot of Mt. Maruyama near Sapporo in Spring, and preserved in pots containing soil till the middle of September (soil temperature 17–20°C) when the root-tips were fixed. Observations of somatic complements were all based on chloralised root-tip cells, which were prepared as follows: Rootlets were first immersed together with rhizomes in 1 percent chloralhydrate (18°C), rinsed in tap-water (13–14°C) for 1 hour respectively, allowed to remain for 2–3 hours on wet filter-paper in a large moistened PETRI dish kept in darkness (19–20°C); then the root-tips were cut and fixed in LA COUR 2BE. All the material was pre-treated under almost the same condition as shown in parentheses. After the ordinary paraffin-section method, they were stained with gentian-violet according to NEWTON's schedule.

1) In the previous papers (HAGA 1934 a, b) the synonymous name *P. quadrifolia* L. var. *obovata* REGEL et TIL. was used.

2) See also GOTOH (1933) and STOW (1935).



Meiosis in PMCs was observed in the middle of May using young flower buds collected directly from their natural habitat, employing BELLING's iron aceto-carminé method.

All drawings were made with an ABBÉ camera lucida using a LEITZ 1/12 oil immersion objective and a LEITZ periblan  $\times 15$  eye piece, giving a magnification of  $\times 1750$  diameters. They were reduced for reproduction to the scale indicated.

### Observations

Before going further, effect of the chloralisation must be inspected because the details of the somatic complements have been based on the treated materials. By the chloralisation the chromosomes are made short and less twisted, but without any conspicuous increase in chromatid diameter. The kinetic constriction becomes very pronounced and elongated, but the long akinetic constriction which separates the trabant from the chromosome bulk remains intact. Thus the influence of chloralisation differs in two functionally and structurally different constrictions (*cf.* Figs. 1-6 with Figs. 15-16 in HAGA 1934a). Further

the chloralised chromosomes do not make a normal metaphase plate (*cf.* HAGA 1935).

The treatment affects the chromosomes in the manner above mentioned, but, as will be seen in Tables 1-9, the relative lengths of the component chromosomes in a complement as well as the relative positions of the constrictions of given chromosomes are not altered. Such an effect is well shown, especially, in the comparison of two complements from one and the same individual which markedly differ in the degree of shortening (*cf.* Tables 7 and 8).

In view of the above findings the following observations will be taken to represent, though in relative values, the natural situations of the chromosome complements. The chromo-



Figs. 1-4. Somatic complements of the four diploid karyotypes. All the chromosomes from one complete metaphase plate are aligned. 1: Type  $CCDD$ . 2: Type  $CC-DD$ . 3: Type  $CCDD^-$ . 4: Type  $CC-DD^+$ .  $\times 1225$ .

some lengths of the complements presented in Figures 1-6 are about 60-70 percent of the lengths measured in non-treated material by the writer (HAGA 1934a). The previous chromosome designations A, B, C, D and E have also been adopted in the present paper.

### 1. Somatic complements of the diploid forms<sup>1)</sup>

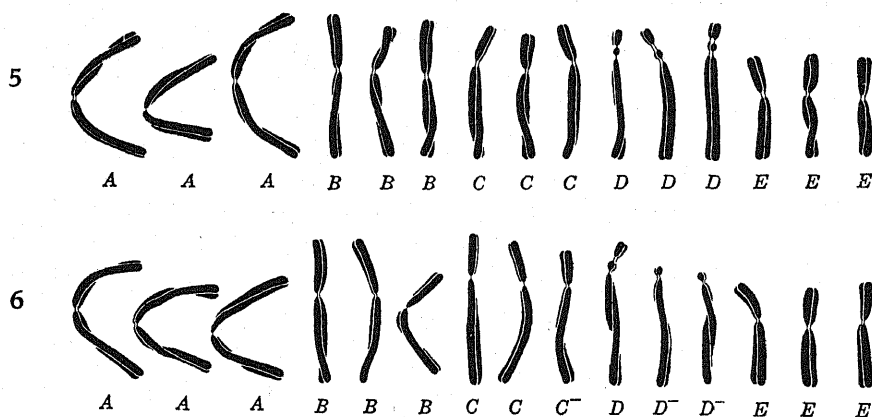
1. Type *C C D D*. All the five pairs are contained in a homozygous condition, representing the unique type of the diploid homozygote<sup>2)</sup> (Fig. 1).

2. Type *C C<sup>-</sup> D D*. One of the *C*-pair is deficient as to its short arm, deficiency amounting to an average 21.5 percent of the normal short arm length. Remaining four pairs are unaltered (Fig. 2).

3. Type *C C D D<sup>-</sup>*. One of the *D*-pair is deprived of its trabant, otherwise entirely normal (Fig. 3).

4. Type *C C<sup>-</sup> D D<sup>+</sup>*. One of the *C*-pair is deficient of its short arm, averaging in deficiency 21.4 percent of the normal short arm length. The length of trabant of one of the *D*-pair is increased, showing about 29.1 percent of its whole length as the surplus length (Fig. 4).

Among the four karyotypes above described the first and the third are the same as those found by GOTOH and STOW (1930) and designated by STOW (1935) as II<sub>2</sub> and II<sub>1</sub> respectively. The other two were found anew by the present study.



Figs. 5-6. Somatic complements of the two triploid karyotypes. All the chromosomes from one complete metaphase plate are aligned. 5: Type *C C C D D D*. 6: Type *C C C D D D*.  $\times 1225$ .

1) The sign — indicates length deficit and + surplus. Previously *D*-chromosome has been designated with *dt* and *D*-chromosome with *d* (HAGA 1934 a, b).

2) The terms homo- and heterozygote were used as to the condition of morphological characters of the pair of chromosomes.

## 2. Somatic complement of the triploid forms

1. Type  $C C C D D D$ . In every chromosome type all three homologues are quite identical with one another in their morphology; apart from the number of homologues there is seen no difference between this and diploid  $C C D D$  type (Fig. 5).

2. Type  $C C C^- D D^-$ . One of the three  $C$ -chromosomes is deficient as to its short arm, the deficiency being 21.4 percent of the normal length of the short arm. Furthermore, two of the three  $D$ -chromosomes are deprived of their trabants (Fig. 6).

The first type is identical with GOTOH and STOW's  $III_3$ , and the second one is also, probably, the same as their  $III_1$  because the material was collected from the same habitat in the botanical garden of our university, where they grew under wild conditions, but they overlooked the deficient  $C$ -chromosome. One triploid form found by the previous investigators ( $III_2$ ) which comprises two  $D$ - and one  $D^-$ -chromosomes instead of three  $D$ 's was missed by the present writer.

## 3. Comparison of the karyotypes

In order to clear up more fully the details of the chromosome complements of the six karyotypes, the writer made precise measurements. To make the technical errors as slight as possible, no special measuring technic was applied, selecting the complete metaphase plate in a single cell revealing the actual lengths, at least, of all the chromosomes composing a haploid set, namely only when they are lying horizontally along their whole lengths. In a majority of cases almost all the components of a complement were measured. The differences in length as well as form percent between the pair were too small to be considered significant, so that only the mean values were tabulated in Tables 1-8, excluding  $C^-$ ,  $D^-$ , and  $D^+$ -chromosome. Degrees of shortening by the chloralisation were shown with lengths in percent of the lengths in non-treated complement (Table 1), which was indicated by S%.

Tables 1-8.<sup>1)</sup> Mean values of the measurements on unaltered chromosomes in a single complement of eight karyotypes

### 1.<sup>2)</sup> $C C D D$

Chromosome	Actual length in $\mu$	L%	F%	S%
<i>A</i>	16.8 + 16.5 = 33.3	26.9	49.5	100.0
<i>B</i>	15.8 + 10.3 = 26.1	21.1	39.5	100.0
<i>C</i>	17.1 + 5.6 = 22.7	18.4	24.7	100.0
<i>D</i>	18.8 + 1.1 + 2.3 = 22.2	17.9	{ 5.0 10.4	100.0
<i>E</i>	11.1 + 8.3 = 19.4	15.7	42.8	100.0
Sum	123.7	100.0	Mean	100.0

1) Position of constriction is indicated by the mark +, and the sum is the whole length of the chromosome. L% and F% indicate length and form percent respectively, the former representing the whole length of a chromosome in percent of total sum of chromosome lengths in a haploid set and the latter

## 2. C C D D

Chromosome	Actual length in $\mu$	L%	F%	S%
A	11.7 + 11.7 = 23.4	27.4	50.0	70.3
B	10.2 + 6.9 = 17.1	20.0	40.4	65.5
C	12.0 + 4.6 = 16.6	19.5	27.7	73.1
D	12.6 + 1.0 + 2.0 = 15.6	18.3	{ 6.4 12.8	70.3
E	7.6 + 5.0 = 12.6	14.8	39.7	64.9
Sum	85.3	100.0	Mean	68.8

## 3. C C-D D

Chromosome	Actual length in $\mu$	L%	F%	S%
A	13.7 + 13.6 = 27.3	28.2	49.8	82.0
B	11.6 + 8.6 = 20.2	20.9	42.6	77.4
C	12.9 + 4.9 = 17.8	18.4	27.5	78.4
D	13.7 + 1.0 + 1.9 = 16.6	17.1	{ 6.0 11.4	74.8
E	8.6 + 6.3 = 14.9	15.4	42.3	76.8
Sum	96.8	100.0	Mean	77.9

## 4. C C D D-

Chromosome	Actual length in $\mu$	L%	F%	S%
A	12.0 + 11.4 = 23.4	27.2	48.7	70.3
B	10.9 + 7.4 = 18.3	21.3	40.4	70.1
C	11.5 + 4.6 = 16.1	18.7	28.6	70.9
D	12.6 + 1.0 + 2.0 = 15.6	18.2	{ 6.4 12.8	70.3
E	7.4 + 5.1 = 12.5	14.6	40.8	64.4
Sum	85.9	100.0	Mean	69.2

5.<sup>3)</sup> C C-D D+

Chromosome	Actual length in $\mu$	L%	F%	S%
A	14.9 + 14.3 = 29.2	27.7	49.0	87.6
B	12.6 + 9.0 = 21.6	20.5	41.7	82.8
C	15.1 + 5.1 = 20.2	19.4	25.2	89.0
D	14.3 + 1.1 + 2.3 = 17.7	16.8	{ 6.2 13.0	79.7
E	9.4 + 6.9 = 16.6	15.7	41.6	85.6
Sum	105.3	100.1	Mean	84.9

the short arm length in percent of the whole length of a chromosome. The upper one of the two form percents of *D*-chromosome represents that of the short arm and the lower that of the trabant.

2) Non-treated complement and the same one given in Table 4 of the previous paper (HAGA 1934 a). Chromosome lengths in this complement were used as the standard lengths for the calculation of S%'s.

3), 4) and 5) In these three complements the long arms of *D*'s are slightly shorter than those of *C*'s. But these mean nothing else than the expectable variations in the contraction grades between two independent chromosomes for the normal relations between *C*- and *D*-chromosome were ascertained repeatedly in the same individuals. The complements in Tables 7 and 8 are taken from the same individual.

## 6. C C C D D D

Chromosome	Actual length in $\mu$	L%	F%	S%
A	14.0 + 13.7 = 27.7	27.5	49.5	83.2
B	12.0 + 8.9 = 20.9	20.8	42.6	80.1
C	13.7 + 5.1 = 18.8	18.7	27.1	82.8
D	14.9 + 1.0 + 2.0 = 17.9	17.8	{ 5.6 11.2	80.6
E	9.2 + 6.1 = 15.3	15.2	39.9	78.9
Sum	100.6	100.0	Mean	81.1

7.<sup>4)</sup> C C C-D D-D- I

Chromosome	Actual length in $\mu$	L%	F%	S%
A	10.9 + 10.6 = 21.5	28.1	49.3	64.6
B	9.1 + 6.9 = 16.0	20.9	43.1	61.3
C	11.1 + 3.9 = 15.0	19.6	26.0	66.1
D	10.6 + 0.7 + 1.4 = 12.7	16.6	{ 5.5 11.0	57.2
E	6.3 + 5.1 = 11.4	14.9	44.7	58.8
Sum	76.6	100.1	Mean	61.6

8.<sup>5)</sup> C C C-D D-D- II

Chromosome	Actual length in $\mu$	L%	F%	S%
A	8.5 + 8.2 = 16.7	28.9	49.1	50.2
B	6.8 + 5.0 = 11.8	20.4	42.4	45.2
C	8.0 + 3.3 = 11.3	19.6	29.2	49.8
D	7.7 + 0.7 + 1.4 = 9.8	17.0	{ 7.1 14.3	44.1
E	4.8 + 3.4 = 8.2	14.2	41.5	42.3
Sum	57.8	100.1	Mean	46.3

As a matter of fact, the chromosome is not an absolute thing but a relative one, so that though the relative values are highly stable, they also vary within a small range. Here it is important to note that the writer has found in the statistical data on one and the same individual of *Trillium kamtschaticum* that the absolute lengths and the form percents of a given chromosome type vary far more than the extent found between the same chromosome types of the different karyotypes of this plant (cf. HAGA 1934 a). Moreover it must be emphasized that the shorter the chromosome or the chromosome part relatively the greater is the observational and measuring error. The variation ranges of absolute lengths in different karyotypes, which are inspectable most simply with S%, do not extend over the range found between two different complements in the same individual (cf. Table 7 and 8).

Thus, if one makes a careful comparison between the results in Tables 1-9, he will be convinced that all the unaltered chromosomes included in six karyotypes are identical and remain unaffected by the genotypic change which may necessarily be caused by the structural hybridity. Averaging up the data in Tables 1-8, mean values of the normal haploid set were obtained, which are presented in Table 9 and diagrammatically in Figure 7.

Table 9. Averaging the results in Tables 1-8

Chromosome	Actual length in $\mu$	$\sigma$	L%	$\sigma$	F%	$\sigma$
A	12.8 + 12.5 = 25.3	$\pm 4.8031$	27.6	$\pm 0.6072$	49.4	$\pm 0.4016$
B	11.1 + 7.9 = 19.0	$\pm 3.9850$	20.7	$\pm 0.3921$	41.6	$\pm 1.2344$
C	12.7 + 4.7 = 17.4	$\pm 3.2280$	19.0	$\pm 0.5037$	27.0	$\pm 1.4866$
D	13.2+1.0+1.9 = 16.1	$\pm 3.4467$	17.6	$\pm 0.6354$	6.2	$\pm 0.6113$
E	8.1 + 5.7 = 13.8	$\pm 3.2187$	15.1	$\pm 0.5012$	11.8	$\pm 1.2654$
					41.3	$\pm 1.5696$
Sum	91.6	$\pm 18.5545$	100.0			

Now we are for a comparison of the structural changes which are found in different karyotypes. The following data were based on the comparison of a pair of normal and changed chromosome that were measured together in one and the same metaphase plate where the two were lying in a horizontal plane along their whole lengths. The measurements were carried out on about ten pairs in each case, resulting as in Tables 10 and 11. With regard to the long arm there was no length difference between altered and unaltered chromosomes, notwithstanding the chromosome type concerned. So no special mention will be made in this respect.

Table 10. Comparison of C-chromosomes in three karyotypes

Karyotype	Deficiency	$\sigma$	F%	$\sigma$
C C-D D	21.5	$\pm 1.1510$	23.1	$\pm 0.1000$
C C-D D <sup>+</sup>	21.4	$\pm 1.5732$	22.8	$\pm 1.0000$
C C C-D D-D-	21.4	$\pm 2.1954$	22.8	$\pm 1.0165$
Mean	21.4	$\pm 1.8253$	22.9	$\pm 0.8005$

In Table 10 the deficit length of the short arm of the C-chromosome was represented in percent of the normal short arm length. Deficiency and form percent of the C-chromosomes which were found in three karyotypes C C-D D, C C-D D<sup>+</sup> and C C C-D D-D- were almost in agreement with one another (Table 10). This suggests significantly the common origin of the C-chromosomes which is comprised, at present, in three different karyotypes.

The measurements showed 29.1 percent ( $\sigma = \pm 2.1221$ ) of the whole length of the trabant of D<sup>+</sup>-chromosome to be the surplus length. A comparison of the length percents

Table 11. Comparison of changed parts of the altered chromosomes with one another and with the corresponding parts of normal ones

L%	of	Mean	$\sigma$
Normal short arm of C		5.1	$\pm 0.3518$
Length of trabant of D		2.1	$\pm 0.2031$
Deficit length of short arm of C-		1.1	$\pm 0.0294$
Surplus length of trabant of D <sup>+</sup>		0.9	$\pm 0.0290$

of the deficit length of  $C^-$ -chromosome and the surplus one of the  $D^+$ -chromosome revealed the interesting fact that the two values are almost agree with one another (Table 11). Thus it seems, though it may be somewhat premature to state it, that translocation of a small segment from the short arm of  $C$ -chromosome to the distal end of the

trabant of  $D$ -chromosome may be the origin of  $C^-$ - and  $D^+$ -chromosome. Of course other interpretations are not impossible, however, the above mentioned seems the most favorable for the circumstances found.

The fact that the long arm lengths of  $D$ - and  $D^-$ -chromosome are equal and the close comparison of the results in Tables 1-11 indicates that  $D^-$ -chromosome has been derived, very probably, from simple deletion of the entire part of the trabant of the  $D$ -chromosome.

From the above results three altered chromosomes  $C^-$ ,  $D^+$  and  $D^-$  were represented diagrammatically in Figure 7, contrasting to the unaltered original ones.

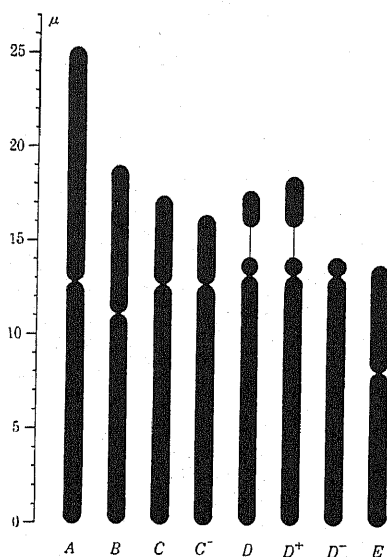


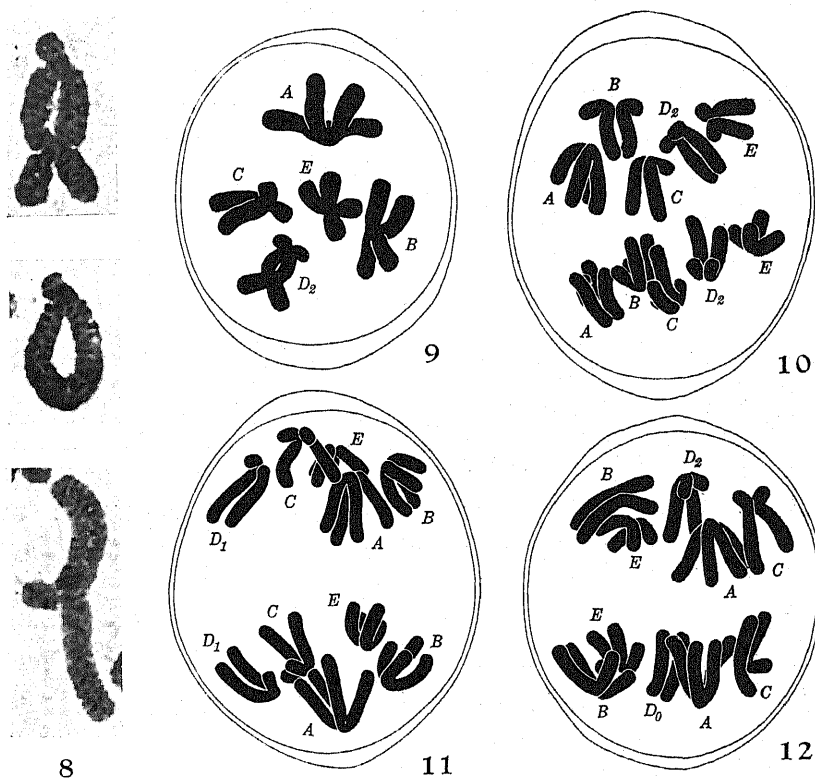
Fig. 7. Diagrammatic representation of the results in Tables, 9, 10 and 11.

#### 4. Meiosis in diploids

Behavior during the prophase was not studied, but through all four karyotypes the meiosis occurs normally except for a somewhat frequent occurrence of non-pairing of one or two pairs, fragmentation and bridge-formation at metaphase and anaphase.

Metaphase pairing was regular, showing 5 bivalents paired at the region of kinetochore and rarely with additional interstitial chiasma (Figs. 8-9). Pairing of the heteromorphic pairs in heterozygous forms is not interrupted by the structural dissimilarities between the partners. Heteromorphic constitution was very clear in the  $D-D^-$  bivalent with the repulsion between the heteromorphic short arms in the plane of reduction (Fig. 8), but it was indiscernible in  $C-C^-$  and  $D-D^+$  bivalents for the minute differences between the partners.

Meiotic processes from first to second anaphase and pollen forma-



Figs. 8-12. Meiotic first divisions in PMCs. 8: Heteromorphic  $D-D^-$  bivalents at first metaphases. Large satellited short arm is seen at the upper left and non-satellited short arm as a small head at the upper right in all three photos. Chiasma in the uppermost is interstitial, in the middle almost terminal and in the lowest is not formed or has slipped off till the metaphase.  $\times 1463$ . (Prof. H. MATSUURA photo). 9-12: Subscript numerals under the  $D$ -type chromosomes indicate number of trabsants. 1 and 2 representing the presence of one and two trabsants respectively and 0 the absence of any. 9: Metaphase in type  $CCDD$ , showing  $A$ -bivalent two chiasmata at both sides near the kinetochore, and  $D$ -bivalent one interstitial chiasma. 10: Anaphase in type  $CCDD$ . 11-12: Anaphases in type  $CCDD^-$ , segregating  $D-D^-$  bivalent equationally as to its short arm in Fig. 11, and reductionally in Fig. 12.  $\times 870$ .

tion are also regular (Figs. 10-15). With  $D-D^-$  bivalent it was ascertained that the heteromorphic pair segregates reductionally as well as equationally at first anaphase, at least, as to its short arms (Figs. 11-12). If it segregates reductionally at first anaphase, then equationally at the second one, and *vice versa* (Figs. 14-15). The ratio reductional to equational segregation in 253 PMCs was 1.0: 5.3, diverging from the ratios hitherto known with a moderate preponderance of the equational one (*cf.* HUSKINS and SPIER 1934 and MATHER 1935).



### 5. Meiosis in triploids

Details before metaphase were not studied. In general meiosis proceeds as in many other autotriploid plants, showing no difference

between the two karyotypes. Irregularities as those observed in diploids occur more frequently in triploids.

At metaphase 0-5 trivalents are seen, with a formation of 5 trivalents prevailing (Fig. 16).<sup>1)</sup> The mode of pairing is the same as in diploids except the number of homologues (Fig. 16). The heteromorphic constitution of the  $D-D-D^-$  trivalent was also clear with the repulsion between the heteromorphic short arms in the planes of reduction (Fig. 16). At first anaphase three homologues paired in a



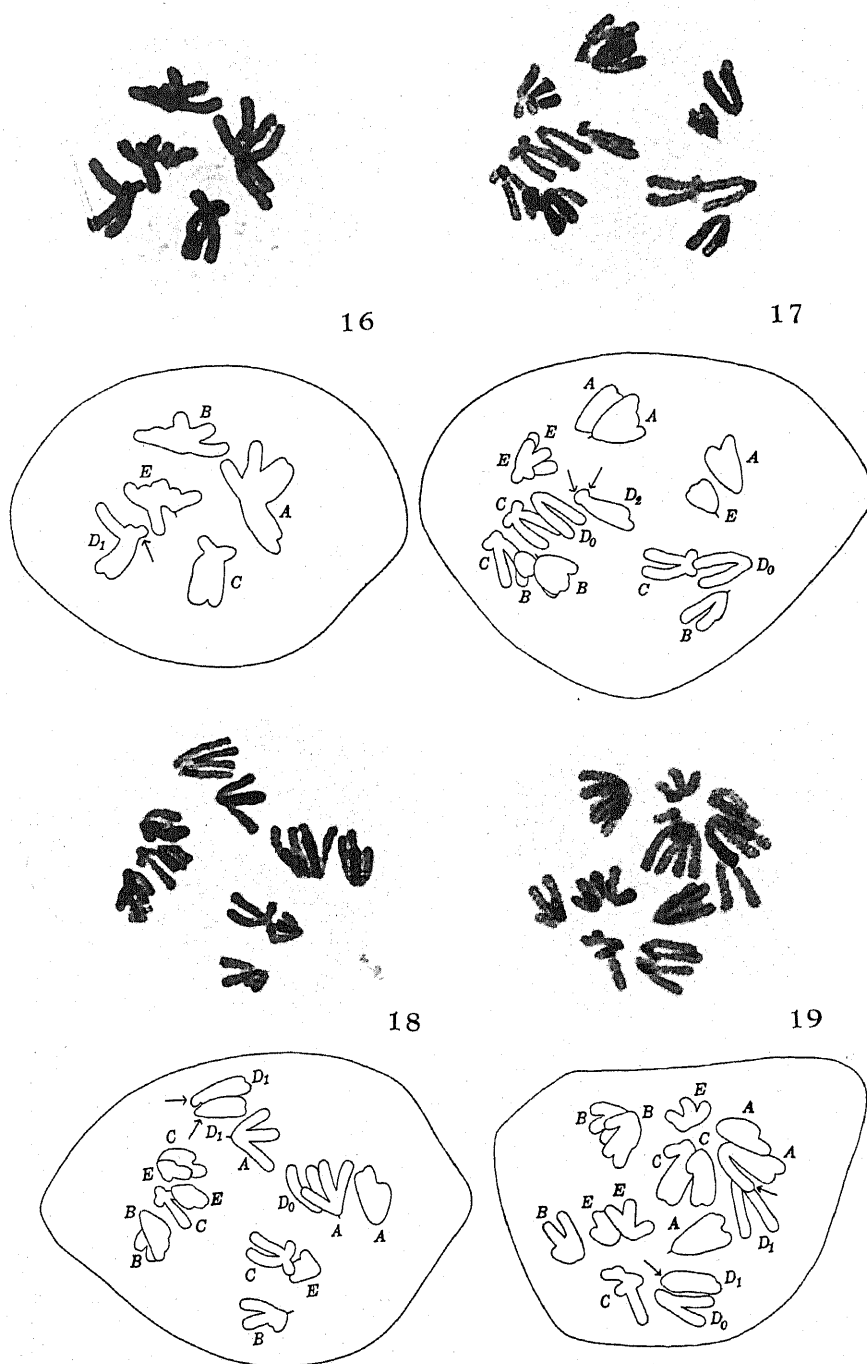
Figs. 13-15. Alinements of meiotic second anaphase chromosomes in two diploid karyotypes, being two divisions in a PMC shown separately at both sides of the Figure number. Daughter halves are faced up and down. Numerals beside the  $D$ -type chromosome indicate the presence (1) or absence (0) of a trabant. 13: Type  $CCDD$ . 14-15: Type  $CCDD^-$ , showing in Figure 14 an equational segregation of the short arm of  $D$ -type chromosomes, and in Figure 15 reductional segregation of them.  $\times 870$ .

trivalent separate from one another and migrate to the poles (Figs. 17-19).

To make clear the mode of anaphase segregation 256 anaphase PMCs of the triploid form  $CC C^-D D^-D^-$  were studied, selecting only those which were believed to have been derived from the metaphases formed of 5 trivalents.<sup>2)</sup>

1) For details confer HAGA (1937).

2) In this plant meiotic chromosomes are easily identifiable as in somatic complement (cf. Figs. 1-22). Thanks to this quality the metaphase pairing was able to deduce with reliable certainty, from a given disposition of the anaphase chromosomes (cf. Figs. 10-12 and 17-19).



Figs. 16-19. Meiotic first divisions in PMCs of the triploid form  $C C C-D D-D-$ . 16: Metaphase showing three trivalents. 17-19: Anaphases, being the chromosome assortments 5-10, 6-9 and 7-8 respectively. For the details compare the photos with the outline figures given below each photo. (Prof. H. MATSUURA Photo.)  $\times 747$ . Outline figures: Subscript numerals under *D*-type chromosomes indicate the number of trabants, 1 and 2 representing the presence of one and two trabants respectively and 0 the absence of any. Arrows indicate the position of trabants.

Table 12. Assortment of chromosomes in 256 meiotic first anaphase PMCs of the triploid form  $C C C-D D-D$ 

Chromosome assortment	5-10	6-9	7-8	Total
Frequency (PMCs)	18	81	157	256
Ratio observed	1.1250	5.0625	9.8125	16.0000
Ratio expected	1.0000	5.0000	10.0000	16.0000
Deviation	+0.1250	+0.0625	-0.1875	0.0000
P.E.	$\pm 0.1633$	$\pm 0.3126$	$\pm 0.3265$	—

Table 13.<sup>1)</sup> Classification of the chromosome assortments given in Table 12 as to their chromosome combinations

## Assortment 5-10.

$A B C D E$	$A B C D E$	PMCs	Ratio obs.	Ratio exp.	Deviation
$(1+1+1+1+1)$	$(2+2+2+2+2)$	18	1.1250	1.0000	+0.1250
Sum		18	1.1250	1.0000	+0.1250

## Assortment 6-9.

$A B C D E$	$A B C D E$	PMCs	Ratio obs.	Ratio exp.	Deviation
$(2+1+1+1+1)$	$(1+2+2+2+2)$	14	0.8750	1.0000	-0.1250
$(1+2+1+1+1)$	$(2+1+2+2+2)$	15	0.9375	1.0000	-0.0625
$(1+1+2+1+1)$	$(2+2+1+2+2)$	15	0.9375	1.0000	-0.0625
$(1+1+1+2+1)$	$(2+2+2+1+2)$	20	1.2500	1.0000	+0.2500
$(1+1+1+1+2)$	$(2+2+2+2+1)$	17	1.0625	1.0000	+0.0625
Sum		81	5.0625	5.0000	+0.0625

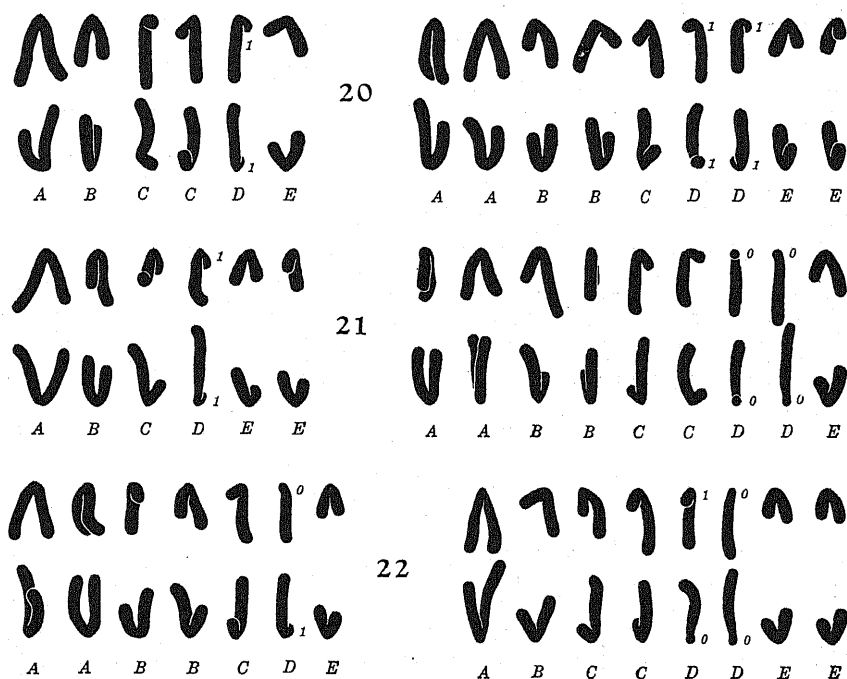
## Assortment 7-8.

$A B C D E$	$A B C D E$	PMCs	Ratio obs.	Ratio exp.	Deviation
$(2+2+1+1+1)$	$(1+1+2+2+2)$	11	0.6875	1.0000	-0.3125
$(2+1+2+1+1)$	$(1+2+1+2+2)$	15	0.9375	1.0000	-0.0625
$(2+1+1+2+1)$	$(1+2+2+1+2)$	15	0.9375	1.0000	-0.0625
$(2+1+1+1+2)$	$(1+2+2+2+1)$	17	1.0625	1.0000	+0.0625
$(1+2+2+1+1)$	$(2+1+1+2+2)$	22	1.3750	1.0000	+0.3750
$(1+2+1+2+1)$	$(2+1+2+1+2)$	15	0.9375	1.0000	-0.0625
$(1+2+1+1+2)$	$(2+1+2+2+1)$	19	1.1875	1.0000	+0.1875
$(1+1+2+2+1)$	$(2+2+1+1+2)$	11	0.6875	1.0000	-0.3125
$(1+1+2+1+2)$	$(2+2+1+2+1)$	13	0.8125	1.0000	-0.1875
$(1+1+1+2+2)$	$(2+2+2+1+1)$	19	1.1875	1.0000	+0.1875
Sum		157	9.8125	10.0000	-0.1875

Merely as to the chromosome number three types of assortment will be expected from the 5 trivalents metaphases, namely 5-10

1) Two anaphase groups in a PMC are shown with the two formulas in parentheses combined with —. The probable error for each case is the same, being  $\pm 0.16327$ . The total sum of the deviations is 0.0000.

(Fig. 17), 6-9 (Fig. 18) and 7-8 (Fig. 19) and their ratio of occurrence must be 1:5:10. This expectation was proved indisputably in the observation (Table 12). To inspect more closely the mode of assortment, every one was classified regarding at the same time the number and combination of chromosomes. Thus the numerical assortment 5-10, 6-9 and 7-8 will be subdivided into 1, 5 and 10 cases respectively as to the combination of chromosomes. And every combination is expected to happen with equal chance. This was also perfectly substantiated (Table 13). Consequently of the pollen grains resulting from 5 trivalents metaphase 3.1 percent will be normal haploid with one complete genom and the same percent diploid with two complete genomes, all other grains containing a complete genom and 1-4 extra chromosomes.



Figs. 20-22. Alinements of meiotic second anaphase chromosomes in two triploid karyotypes, being the two divisions in a PMC shown separately at both sides of the Figure number. Daughter halves are faced up and down. Numerals beside the *D*-type chromosomes indicate the presence (*i*) or absence (*o*) of a trabant. 20: Type *CCCCDD*, being the chromosome assortment 6-9. 21-22: Type *CCC-DDD*, being the chromosome assortment 6-9 and 7-8 respectively. Segregation of the short arm of *D*-type chromosomes is equational in Fig. 21 and reductional in Fig. 22.  $\times 870$ .

Heteromorphic trivalent  $D-D-D^-$  segregates at first anaphase, as in bivalent  $D-D^-$ , reductionally as well as equationally (Figs. 17-19). Their second anaphase segregation is reductional or equational

according to the previous one (Figs. 20-22). The ratio of reductional to equational segregations is not determined, however it was apparent that equational segregation is prevailing as in bivalent  $D-D^-$ .

### Discussion

1. **Origin of the karyotypes.** The homozygous diploid form was represented only by  $C C D D$  type which spread most abundantly in the natural population, so that it seems highly pertinent to consider this type to be the prototype of the other ones. Remaining heterozygous diploids are considered to have been derived from the mating of a normal with an exceptional gamete containing a simple translocation or simple deletion, or between the latter ones.

Through all karyotypes found there were no structural changes in chromosomes  $A$ ,  $B$  and  $E$ .  $C^-$ -chromosomes observed in two diploids  $C C^-D D$ ,  $C C^-D D^+$  and in one triploid karyotype  $C C C^-D D^-$  have showed the same deficiency of short arm length, strongly suggesting a common origin for them.  $D^-$ -chromosome is obviously the result of simple deletion of the trabant of  $D$ -chromosome. The surplus length of the trabant of a  $D^+$ -chromosome is almost equal to the deficit length of the short arm of a  $C^-$ -chromosome. From these facts one may safely conclude that the simple translocation of a small segment from the short arm of  $C$  to the distal end of the trabant of a  $D$ -chromosome to be the very cause of origin of  $C^-$ - and  $D^+$ -chromosome. However the expected multivalent association  $C^-C-D^+D$  such as has been demonstrated in the case of *Rumex* (YAMAMOTO 1935) was not met with at the meiosis of a heterozygous diploid  $C C^-D D^+$ . But, it seems highly improbable that this is decisive disproof for the above interpretation because such a circumstance is of the most expectable in considering only about 1 micron of the somatic length of the translocated segment. A similar case of frequent non-pairing between the homologous parts was demonstrated experimentally in the translocation from  $A$ - to  $C$ -chromosome of *Crepis capillaris* (PETROV 1935).

As to the origin of the structural change of the chromosomes environmental factors must be responsible for it, among them the influence of temperature may be the most common and effective in nature. Recent experiments with X-radiation (LEWITSKY *et al.* 1934, CATCHESIDE 1935, and MATSUURA and HAGA 1937), several chemicals (LEWITSKY and ARARATIAN 1931), aging (GERASSIMOVA 1935), etc., have provided many instances for such chromosome alterations. Interestingly it has been confirmed that experimental effects exerted upon various stages of development, *e.g.*, upon seeds, young shoots, PMCs or pollen grains, are equally effectual in the formation of

karyotypic aberration. So when and where the aberrant karyotypes of this plant have originated is undeducible, especially in the habit of this plant with a perennial rhizome which bears flower buds for the next Spring already in the previous Autumn and with very poorly germinating seeds. The only sign is seen in the somewhat high frequency of abnormal meiosis showing fragmentation and chromatid bridge, which indicate essentially the possibility of the occurrence of deletion and translocation (*cf.* MATSUURA and HAGA 1937).

Triploids are explained, as in many other autotriploids, as the results of fusion between a haploid and a diploid gamete. The karyotype found in triploids may also be explicable as in diploids, but with some complexity.

Formation of diploid gamete in diploids probably has been caused by environmental influences, especially by temperature as in the experimental cases in *Liriope* and *Scilla* (SHIMOTOMAI 1927), in *Lychnis* (TAKAGI 1928), etc., but not excluding the possibilities by polyploidal chimera (TAHARA 1933), by the action of certain genes (BERGNER *et al.* 1934 and SATINA and BLAKESLEE 1935) or by phytopathological infection (KOSTOFF 1933), etc.

Though it is a secondary process, the production of gametes containing two complete genomes by an autotriploid, which has been definitely shown in the present study, must be added here. Such a function of autotriploids may have an important rôle in the evolution of allo- as well as autopolyploidy as proved partially in some cases, *e.g.*, in *Crepis* (NAVASHIN 1931), in *Rumex* (ONO 1935), etc.

Homozygous plants with regard to  $C^-$ ,  $D^+$ - and  $D^-$ -chromosome are not found by GOTOH and STOW nor by the writer as yet. What is the reason for this? One may expect the governing of the lethal mechanism as in *Galtonia* (NAWASCHIN 1927), *Matthiola* (PHILP and HUSKINS 1931), *Petunia* (MALINOWSKI 1935), etc. The writer wishes fully to deal with these problems upon a later occasion.

**2. Plant-geographical.** Recent works by HAGERUP (1932 and 1933) and MATSUURA (1935) have revealed several instances in which polyploids enter into a new territory on account of change in their biological properties, resulting in a distributional differentiation between polyploids and the original form. For example in *Fritillaria camchatcensis* the diploid form was restricted to the alpine region, whereas the triploid grows only in low lands (MATSUURA 1935).

The case in *Paris hexaphylla* was in striking contrast to the above instances, diploid and triploid populations growing in contact with one another in their natural haunt. Such also occurred within the karyotypes of diploids and of triploids (Fig. 23). Diploid  $C\ C-D\ D$

and triploid  $C C C^{-} D D^{-} D^{-}$  were found a little apart in the area covered by the map shown in Text-figure 23. This offers further evidence for the above interpretation on the origin of karyotypes.

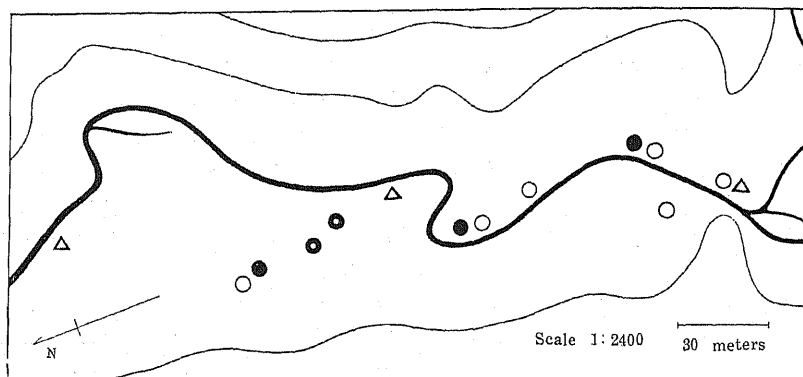


Fig. 23 A contour-line map along the little stream running at the foot of Mt. Maruyama near Sapporo, showing the occurrence of the four different karyotypes in their natural haunt.  $\circ$ : Type  $CCDD$ .  $\bullet$ : Type  $CC-DD^{+}$ .  $\bullet$ : Type  $CCDD^{-}$ .  $\triangle$ : Type  $CCDDDD$ .

Such a condition will be interpreted with most certainty to the effect that polyploid as well as karyotypic alteration found in this plant has insufficient effect in the change of biological properties to cause a distributional or ecological differentiation.

### 3. Meiotic segregation of the heteromorphic chromosome pair.

On account of their significance for an interpretation of meiosis in relation to some genetic phenomena, the behavior of heteromorphic chromosome pairs has been studied by several investigators, but resulting in very diverse conclusions with different materials (*cf.* HUSKINS and SPIER 1934, and MATHER 1935). In *Triticum vulgare* HUSKINS and SPIER made an interpretation for the mechanism of equational and reductional segregation of the heteromorphic bivalents in relation to chiasmotype theory. Also MATHER had made a theoretical consideration on this problem, advancing the 2:1 ratio of equational to reductional segregation as an "equilibrium proportion" on the basis of the same theory.

In the present material heteromorphic  $D-D^{-}$  bivalents segregated with a ratio of 1.0 reductional to 5.3 equational as to its heteromorphic short arms. This ratio is not expectable directly from MATHER's formula. According to him such a proportion "will depend on the mean and variance of the chiasma frequency distribution" on the short arm. However, in such a bivalent which retained paired at metaphase very probably at the kinetochore as in *Trillium kamtschaticum*, the behavior of the kinetochore must be taken in consideration

when analysing the problem (cf. MATSUURA 1937). The writer hopes to leave the final conclusion to the time when other statistical data are available.

**4. Segregation of trivalents.** There are advanced many hypotheses of the karyokinetic mechanism (cf. BLEIER 1931 and KOLLER 1934). Among them, though some parts are objectionable, BLEIER's hypothesis seems to be the most elaborated one, interpreting many special modes of karyokinesis with consistent principles carried out by the cooperation of autonomous movement of chromosomes, repulsion between split halves or components of a paired chromosomes, spindle bipolarity and the division of spindle substance.

According to BLEIER, at metaphase three kinetochores of a trivalent mutually repulse each other potentially if they are not separated until the anaphase and visibly if they separated already before the metaphase, in the latter case the pairing being retained only by chiasmata (Fig. 24). The former case is represented by the present material and the latter is known in many other plants. At metaphase trivalents are subjected to the influence of spindle bipolarity, forming a metaphase plate. In equatorial plane, BLEIER's "Gleichgewichtszone", three kinetochores of a trivalent must be disposed in principle, to attain a metaphase-anaphase equilibrium as shown in Text-figure 24 by the cooperation of repulsion between the three kinetochores and the bipolarity of spindle. Although it is a special case one may indisputably prove such a disposition in a tripartite sex chromosome such as in *Rumex* (cf. ONO 1935 and YAMAMOTO 1935).

At anaphase the kinetochores separate if unseparated before that time, and chromatids within a chromosome repulse one another, if they are formed, cancelling all chiasmata. After this stage the chromosomes will behave as those derived from the bivalents, kinetochore *a* and *b* going pole  $P_1$  and *c* to  $P_2$  (Fig. 20). Thus the anaphase segregation of a trivalent is regular determined by the metaphase disposition of the three kinetochores, in other words, it is not determined by the so-called random distribution of the extra member.

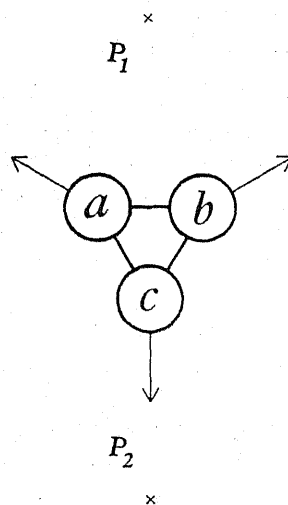


Fig. 24. A diagrammatic representation of the metaphase equilibrium of a trivalent, indicating  $P$  poles, *a*, *b*, and *c* kinetochores which repulsing one another in the direction shown by arrows.



However it is evident that the metaphase disposition of three kinetochores is determined by chance, *i.e.* the position of *c* in Text-figure 24 may be occupied also by *a* or *b* in equal chance with *c*. And it is independent between the trivalents. So self-evidently only gametes with  $n-2n$  chromosomes comprising at least a complete haploid set must result from metaphases with the trivalents corresponding to the basic chromosome number  $n$ . The present study has offered conclusive evidence for this.

### Summary

1) Six karyotypes were distinguished in *Paris hexaphylla* CHAM. which has a basic chromosome number 5 consisting of morphologically distinguishable chromosomes *A*, *B*, *C*, *D* and *E*. Four of them were diploid and two triploid:

Diploids ( $2n = 10$ )			Triploids ( $2n = 15$ )		
Karyotype	1	$C C D D$	Karyotype	1	$C C C D D D$
"	2	$C C^- D D$	"	2	$C C C^- D D^-$
"	3	$C C D D^-$			
"	4	$C C^- D D^+$			

Karyotypic aberration concerns only chromosomes *C* and *D*.  $C^-$  and  $D^+$ -chromosome were suggested as having resulted from simple translocation from *C* short arm to trivalent of *D*.  $D^-$ -chromosome was interpreted as originated from a simple deletion of the whole trivalent of *D*. Except the homozygous diploid  $C C D D$  and the triploid  $C C C D D D$  all other karyotypes were heterozygous as to  $C^-$  or  $D^-$ -type chromosome or both of them.

2) There was no distributional or ecological difference between diploids and triploids and between the karyotypes, showing that polyploidy and the karyotypic changes found in this plant are not sufficiently effectual to cause a biological differentiation.

3) The heteromorphic constitutions of  $D-D^-$  bivalents and  $D-D^-D^-$  trivalents were easily discerned at meiosis in PMCs. Heteromorphic short arm segregates equationally as well as reductionally at first anaphase. The ratio of reductional to equational segregation was 1.0 : 5.3 in  $C C D^- D$  diploid.

4) Metaphase-anaphase behavior of the trivalents was discussed on the basis of observations on anaphase segregation of 5 trivalents metaphases. Anaphase behavior of the trivalent is a regular one for itself, segregating 1 to 2 chromosomes. Thus from 5 trivalents metaphases only the gametes with 5-10 chromosomes comprising at least a complete haploid set must result. This was really verified in the present study.

It is the writer's pleasure to express his sincere thanks to Professor H. MATSUURA under whose valuable suggestions and encouragement the present work was carried out.

#### Literature Cited

- Bergner, A. D., Cartledge, J. L. and Blakeslee, A. F. 1934. Chromosome behavior due to a gene which prevents metaphase pairing in *Datura*. *Cytologia* 6: 19-27.
- Bleier, H. 1931. Zur Kausalanalyse der Kernteilung. *Genetica* 13: 27-76.
- Catcheside, D. G. 1935. X-ray treatment of *Oenothera* chromosomes. *Genetica* 17: 313-341.
- Gerassimova, H. 1935. The nature and causes of mutations II. Transmission of mutations arising in aged seeds: Occurrence of "homozygous dislocants" among progeny of plants raised from aged seeds. *Cytologia* 6: 431-437.
- Gotoh, K. und Stow, I. 1930. Karyologische Studien an *Paris* und *Trillium* (Vorl. Mitt. in Japanisch). *Japan. Journ. Gen.* 5: 114-117.
- 1933. Karyologische Studien an *Paris* und *Trillium*. *Japan. Journ. Gen.* 8: 197-203.
- Haga, T. 1934a. The comparative morphology of the chromosome complement in the tribe *Parideae*. *Journ. Fac. Sci. Hokkaido Imp. Univ. Ser. V.* 3: 1-32.
- 1934b. On the karyotypes and their gametes of *Paris quadrifolia* L. var. *obovata* REGEL et TIL. (Prelim. note in Japanese with Engl. Résumé). *Bot. Mag. (Tokyo)* 48: 241-248.
- 1935. On nuclear polymorphism in *Paris hexaphylla* CHAM. (Prelim. note in Japanese). *Japan. Journ. Gen.* 11: 286-287.
- 1937. Genom and polyploidy in the genus *Trillium*. *Japan. Journ. Gen.* (In the press).
- Hagerup, O. 1932. Über Polyploidie in Beziehung zu Klima, Ökologie und Phylogenie. Chromosomenzahlen aus Timbktu. *Hereditas* 16: 19-40.
- 1933. Studies on polyploid ecotypes in *Vaccinium uliginosum* L. *Hereditas* 18: 122-128.
- Huskins, C. L. and Spier, J. D. 1934. The segregation of heteromorphic homologous chromosomes in pollen mother-cells of *Triticum vulgare*. *Cytologia* 5: 269-277.
- Koller, P. C. 1934. The movements of chromosomes within the cell and their dynamic interpretation. *Genetica* 16: 447-466.
- Kostoff, D. 1933. A contribution to the sterility and irregularities in the meiotic processes caused by virus diseases. *Genetica* 15: 1.3-114.
- Kuhn, E. 1928. Zur Zytologie vom *Thalictrum*. *Jahrb. wiss. Bot.* 68: 332-430.
- Lesley, M. M. and Lesley, J. W. 1935. Heteromorphic A chromosomes of the tomato differing in satellite size. *Genetics* 20: 563-580.
- Lewitsky, G. A. and Araratian, A. G. 1931. Transformations of chromosomes under the influence of X-rays. *Bull. Appl. Bot. Gen. Pland Breed.* 27: 265-303.
- Lewitsky, G., Shepeleva, H. and Titova, N. 1934. Cytology of  $F_1$ ,  $F_2$  and  $F_3$  of X-rayed *Crepis capillaris* WALLR. *Plant Industry USSR* 11: 3-9.
- Malinowski, E. 1935. Studies on unstable characters in *Petunia*. I. The extreme flower type of the unstable race with mosaic color patterns. *Genetics* 20: 342-356.
- Mather, K. 1935. Reductional and equational separation of the chromosomes in bivalents and multivalents. *Journ. Gen.* 30: 53-78.
- Matsuura, H. 1935. On karyo ecotypes of *Fritillaria camschatcensis* (L.) KER-GAWLER. *Journ. Fac. Sci. Hokkaido Imp. Univ. Ser. V.* 3: 219-232.
- 1937. Chromosome studies on *Trillium kamtschaticum* PALL. III. The mode of chromatid disjunction at the first meiotic metaphase of the PMC. *Cytologia* 8: 142-177.
- and Haga, T. 1937. Do. VI. The effects of X-radiation upon the meiotic chromosomes. (In preparation).

- Medwedewa, G. B. 1929. Über die „Trabanten“ bei *Crepis dioscoreidis* L. (Vorläuf. Mitt.) *Zeits. Zellf. mikroskop Anat.* 10: 150-163.
- Navashin, M. 1933. On the chromatin deficiency in *Crepis* leading to partial sterility and to forming of a heteromorphic chromosome pair. *Z. I. A. V.* 63: 218-223.
- Nawaschin, S. 1912. Zellkerndimorphismus in somatischen Zellen von *Galtonia candicans*. *Bull. Acad. Sci. St. Petersburg* 6: 373-385. (Russian, cited after S. Nawaschin 1927 and M. Navashin 1933).
- 1927. Zellkerndimorphismus bei *Galtonia candicans* DES. und einiger verwandten Monokotylen. *Ber. Deutsch. Bot. Gesel.* 45: 415-428.
- Ono, T. 1935. Chromosomen und Sexualität von *Rumex acetosa*. *Sci. Rep. Tōhoku Imp. Univ. 4 Ser. Biol.* 10: 41-210.
- Petrov, D. F. 1935. The reduction division in karyotypical aberrants of *Crepis capillaris* WALLR. *Bull. Appl. Bot. Gen. Plant Breed. Ser. II.* 8: 29-58, 169-183 (Russian with English résumé).
- Philp, J. and Huskins, C. L. 1931. The cytology of *Matthiola incana* R. Br., especially in relation to the inheritance of double flowers. *Journ. Gen.* 24: 359-404.
- Satina, S. and Blakeslee, A. F. 1935. Cytological effects of a gene in *Datura* which causes dyad formation in sporogenesis. *Bot. Gaz.* 96: 521-532.
- Shimotomai, N. 1927. Über Störungen der meiotischen Teilungen durch niedrige Temperatur. *Bot. Mag. (Tokyo)* 41: 147-160.
- Stow, I. 1935. On the correlation between the satellite of chromosome and the leaf shape in *Parideae* from Hokkaido. *Journ. Fac. Sci. Hokkaido Imp. Univ. Ser. V.* 4: 31-46.
- Tahara, M. 1933. Chromosome morphology in *Smilacina japonica*. *Sci. Rep. Tōhoku Imp. Univ. 4 Ser. Biol.* 8: 33-37.
- Takagi, F. 1928. The influence of the higher temperature on the reduction division of the pollen mother cells of *Lychinus sieboldii*, van HOUTTE. *Sci. Rep. Tōhoku Imp. Univ. 4 Ser. Biol.* 3: 461-466.
- Yamamoto, Y. 1935. Karyogenetische Untersuchungen bei der Gattung *Rumex*. IV. Ein Fall von einfacher Translocation bei *Rumex acetosa* L. *Cytologia* 6: 407-412.

**POSTSCRIPT.** With respect to the mode of opening out of the heteromorphic short arms of *D-D*-bivalent, it was stated in the present paper that they always open in the plane of reduction, but extensive reinvestigation newly undertaken revealed the interesting fact that the concerning heteromorphic arms open in the equational (E) as well as in the reductional plane (R). The ratio E:R was 2:1 (actually 202:98 in a total of 300 *D-D*-bivalents), in accordance with the expectation from the Neo-two-plane theory advocated by Professor H. Matsuura\*; in other respects the given data remain unaltered. For further informations readers may refer to a later publication by the present writer.

(T. Haga, May 27, '37).

\* Matsuura, H. 1937. On the chiasma theories for the mechanism of crossing over. *Japan. Journ. Gen.* 13: 41-42 (in Japanese).

## Experimental Researches on the Non-Newtonian Nature of Protoplasm

By

H. Pfeiffer, Bremen

"The ideal college should have three floors—a ground floor for molecular physics, a first floor for biophysics, and a top floor for cell mechanics."

(W. B. HARDY.)

### Introduction

It is a *very great pleasure* to join in the congratulation of Professor KENJIRO FUJII's seventieth birthday, and to pay tribute to this great cytologist who has done so much to advance this science by his own researches as well as by creation of this international journal for all the problems of Cytologics and Genetics in their widest sense. I count myself among those who have been started by his stimulations, and consider it an honour to belong to the standing collaborators of his journal "*Cytologia*". For these reasons, it is to be expected that I contribute a little *paper from Cytophysics* among the festive publications. So I should like to continue another paper on the subject which I have spoken on during the 6th international botanic congress at Amsterdam<sup>1)</sup>. At that time I have already tried to show the elasticity of protoplasm, but the subject matter of the following paper exceeds the statements given there, and adds a new method of demonstrating the elastic behaviour of protoplasm.

### I. The anomalous flow of protoplasm

As is well known a surface tension film can behave like an elastic membrane. Moreover, even in mass, solutions which we ordinarily regard as fluid may have elastic properties in spite of the fact that it flows fairly easily. An usual method of demonstrating the elasticity of colloidal solutions as protoplasm is to measure their *viscosity at various rates of shear*<sup>2)</sup>. Pure liquids and true solutions

1) H. PFEIFFER, Physics (New York) 7, 302 (1936).

2) In addition to the measuring the viscosity at various shearing stresses there are, it is true, *further experimental tests* used for the elasticity of protoplasm. They may be arranged as follows:

a) the osmotic increase and decrease of protoplasmic volume according as the circumstances may require it (C. NÄGELI, S. SCHWENDENER);

b) several gravity methods (A. HEILBRONN, E. BÜNNING, W. O. KERMACK, A. G. M'KENDRICK, E. PONDER);

c) the centrifuge method with varied rotatory speeds (L. V. HEILBRUNN);

have the same viscosity no matter what the rate of shear. They obey POISEUILLE's law and are said to be Newtonian, that is to say, to exhibit true viscous flow. The rate of flow  $v/t$  will change with pressure, but the viscosity  $\eta$  remains constantly. On the other hand, elastic or *non-Newtonian fluids* have a lower viscosity as the rate of shear increases. They possess not *one* viscosity value, but an infinite number of values. We cannot hope here to consider the mathematical features of this difficult problem<sup>1)</sup>. These questions of theory of non-Newtonian fluids are rather out of the range of the Protoplasmatics and must be left to solve to the Rheologists. I content myself with the experiments.

Unfortunately we cannot cause the protoplasm to flow through a capillary. So I use another method which has been employed by FR. VLÈS<sup>2)</sup> with eggs of Tardigrades. The same apparatus has been used several times for measurements of the *surface tension* of protoplasm against a liquid medium<sup>3)</sup> as far as for different experiments of *deformation* of protoplasm including attempts in order to ascertain *moduls* of shearing stress and of extension<sup>4)</sup>.

*Naked protoplasmic drops* were obtained partly from liquefying pericarps (*Physalis Alkekengi*, *Taxus baccata*, *Prunus spinosa*, some *Solanum* species), partly by means of experimental denudation of the protoplasts<sup>5)</sup> within epidermal

d) the determination by means of the electromagnetic attraction of a minute nickel particle embedded in the protoplasm (A. HEILBRONN, H. FREUNDLICH, W. SEIFRIZ);

e) direct observations on rigidity and elastic contraction by means of microdissection (W. SEIFRIZ, G. W. SCARTH, E. PONDER, JANET Q. PLOWE, &c.). There are many points in the behaviour of the plasmalemma which are more readily explained on the assumption of elasticity than of surface tension alone. The behaviour of the mesoplasm against the moved dissection needle suggests that it, too, possesses elasticity in a low degree. But all these observations includes, nevertheless, sometimes a little uncertainty. If protoplasm is elastic, then STOKES' formula is not applicable, and, therefore, neither the gravity nor the centrifuge methods are suitable. Osmotic volume measurements are also not of use for the purpose. So *the best tests are*:

f) the demonstration of anomalous flow (FR. VLÈS, H. PFEIFFER);

g) the direct measurement of the absolute viscosity and its change inversely proportional to the rate of shear (H. PFEIFFER), and

h) the proof of the appearance of a structural arrangement of particles during the protoplasmic flow (by means of polarization microscopy; firstly shown by the author in this paper).

1) W. SEIFRIZ, Protoplasm, p. 225, 229 sq. (New York and London, McGraw-Hill, 1936).—On the extensibility of protoplasm comp. also: SEIFRIZ, Brit. J. exp. Biol. 1, 431 (1924); Protoplasma (Leipzig) 1, 345 (1926), and 9, 177 (1930); J. of Rheol. (New York) 1, 261 (1930), and in collaboration with JANET PLOWE, ibid. 2, 263 (1931).

2) FR. VLÈS, Bull. Instit. Océanogr. Monaco, No. 589 (1931); Arch. zool. exp. et gén. (Paris) 75, 421 (1933).

3) H. PFEIFFER, Protoplasma 25, 397 (1936).

4) H. PFEIFFER, Cytologia (Tokyo) 6, 329 (1935); Protoplasma 26, 372 (1936).

5) H. PFEIFFER, Z. wiss. Mikrosk. (Leipzig) 50, 429 (1934).

cells (onion-skin of *Allium Cepa*) and parenchyma cells (from leaves of *Hyacinthus orientalis*, *Orchis maculatus*). Besides we find useful material by opening the cells of several *Chara* species below liquid paraffin, if need by staining the cells intra-vitam with 0.05 percent chrysoidin. At last I have employed the cells present in saliva (resembling leucocytes) obtained from the not rinsed human mouth.

The apparatus of deformation (fig. 1) consists of a capillary *c*, a water manometer *m*, and a tube *v* with india-rubber aspirator bulb and two-passage tap, all three united by a Y-tube *z* between<sup>1)</sup>. After sucking up the protoplasm by means of a vacuum, measured on the attached manometer, we observe the deformation of the objects. We measure the rate of flow  $v/t$ , that is to say, the time (in sec.) necessary for filling a precise length of the capillary (in  $10^{-4}$  cm.) at any negative pressure (in cm.  $H_2O$  of the manometer).

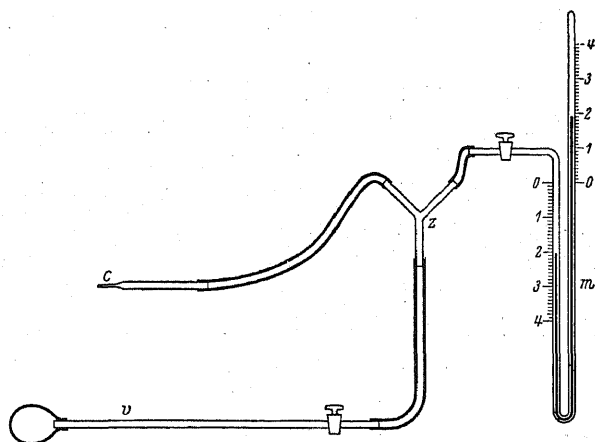


Fig. 1. Apparatus for inducing graded shearing forces.

The curve of the rate of flow  $v/t$

as ordinate plotted against the vacuum head (shearing stress)  $p$  as abscissa exerted on the objects shows a remarkable peculiarity. It is

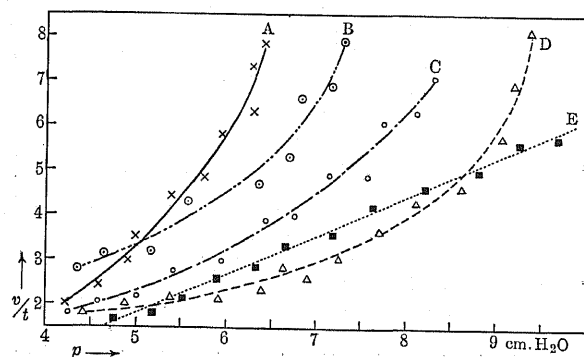


Fig. 2. Curves of the protoplasmic deformation flow rate  $v/t$  plotted against the shearing force  $p$  demonstrated on protoplasmic drops of *Physalis pericarps* (A, C, D) or out from denuded protoplasts of *Orchis maculata* (B) after plasmorrhysis in 0.5 ( $\times$  —  $\times$ ), 0.56 ( $\odot$  —  $\odot$ ), 0.6 ( $\circ$  —  $\circ$ ), and 0.65 mol.  $KNO_3$  ( $\triangle$  —  $\triangle$ ), moreover (D) on inanimate droplets of glycerin ( $\blacksquare$  —  $\blacksquare$ ).

not a straight line, as it would be expected if protoplasm were an ideal liquid, but the velocity of the shearing flow increases with increasing force, at first slowly, later more and more quickly (fig. 2). In experiments with model liquids, the values of the quotients of  $p$  to  $v/t$  are nearly on a straight line if the fluid is inelastic, as in water or glycerin.

1) H. PFEIFFER, Protoplasma 23, 210 (1935).

But there is obtained quite an appreciable curve if the material used shows anomalous flow, as if with sodium stearate. Naked *cells plasmorrhized*<sup>1)</sup> by hypertonic media of different concentrations (salts or non-electrolytes) differ from normal ones, as the slope of the curves decreases the more the plasmorrhysis rate increases. On the other hand, the slope of the curves decreases as the working *temperature* either increases to 25 to 30°C. or decreases to 12 to 15°C. Here we find apparently a relation similar to that of temperature on the viscosity of protoplasm.

The most of these results I have already given in my lecture at Amsterdam. But since that time I have repeated the experiments with other objects and at more shearing stresses on each deformation experiment. Often the protoplasm seemed to possess a *yield value*, i.e. to require an initial force to start flow<sup>2)</sup>. But this phenomenon may be dependent on the adhesion of protoplasm at the capillary wall. As the reality of such a phenomenon in consequence of many experiments of colloid chemists and rheologists is open to controversy, I am in dread of those interpretation all the more. The fact of anomalous flow of protoplasm, however, is shown by means of the course of curves of the rate of flow plotted against the shearing stress.

## II. The absolute viscosity before and after shearing

The viscosity of inelastic or true fluids remains constantly as the pressure is varied. At elastic liquids, however, the *viscosity becomes less and less* as the pressure is increased. So we may demonstrate the non-Newtonian nature of the protoplasm by means of measuring the differences in absolute viscosity the material shows before and after shearing.

If protoplasm is an elastic liquid we cannot use STOKES' formula. So all the conventional methods to measure the protoplasmic viscosity we have to eliminate as unsuitable. Only the *rate of Brownian movement* is adapted for our purpose. A preliminary attempt to calculate the viscosity of protoplasm from the Brownian movement has been made by L. V. HEILBRUNN. At the same time L. G. M. BAAS BECKING, H. v. D. SANDE BAKHUYZEN and H. HOTELLING have considered in more detail the viscosity of establishing a satisfactory estimate

1) Comp. H. PFEIFFER, Cytologia 3, 26 (1931); 4, 52 (1932); 5, 308, 507 (1934); Biodynamica (Normandy) No. 6 (1935); Cytologia 7, 256, 283 (1936).

2) E. G. BINGHAM, Fluidity and plasticity (New York 1922); J. M. BURGER, in: First report on viscosity and plasticity, p. 5, 12 (Amsterdam, Noord-Hollandsche Uitgevers-Maatsch., 1935); C. J. VAN NIEUWENBURG. *ibid.*, p. 141.—The real existence of a *yield value* is rather uncertain, comp. B. RABINOWITSCH, Z. physik. Chem. (Leipzig), A, 145, 1 (1929), and 166, 257 (1933). The phenomenon of the *anomalous or structure viscosity* was, moreover, since H. GARRETT and A. DU PRÉ DENNING often the subject of investigations, especially of W. OSTWALD, W. R. HESS, H. FREUNDLICH, E. HATSCHEK, M. REINER, A. SZEGVARI and of many collaborators.



of protoplasmic viscosity, by the use of A. EINSTEIN's equation for horizontal displacement.

Perhaps the most significant measures from the Brownian movement are those of J. PEKAREK which determine the number of the "mean two-way first passages" of the moved particles<sup>1)</sup>. He finds the absolute values of viscosity from the formula

$$\bar{\eta} = \frac{R}{N} T \cdot \frac{t}{3\pi\alpha l^2 n}$$

$$= \frac{R}{3\pi l^2 N} \cdot \frac{Tt}{\alpha n}$$

(where  $R$  the absolute gas constant,  $N$  the Loschmidt number,  $T$  the absolute temperature,  $t$  the time during the counted passages,  $\alpha$  the radius of the observed particle,  $l$  the distance between two scale lines of the ocular,  $n$  the number of the passages).

Combining this method with the deformation experiments we prefer protoplasts with a relatively thick cytoplasmic surface layer, as from opened cells of *Chara fragilis*. Then we do not need to introduce R. LADENBURG's correction into our equation, and we become certain of observing particles wholly within the cytoplasm. In order to have a size of comparison, we assume the viscosity values through a whole series of increasing heads and connect the values with the initial one.

Changes in cytoplasmic viscosity occur during the influence of shearing forces on the material, the curves as given in fig. 3 bear this out. Under suction the viscosity tends to be less, and as the shearing stress is increased, there is an increase in speed of the Brownian movement. The values of viscosity, as shown on the coordinate scale, are distinctly lower as the rate of shear increases. The fact that a change does occur, and is in the indicated direction, indeed, is fulfillment of the prediction derived from the a priori analysis.

On some results I have already reported to the section of Cytology of the 6th international botanic congress at Amsterdam,

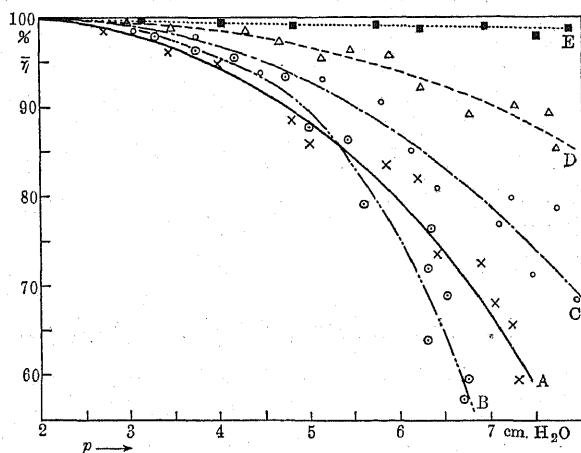


Fig. 3. The absolute viscosity values after influence of shearing forces expressed as percentage of the initial values plotted against the shearing force demonstrated on protoplasmic drops of opened cells of *Chara fragilis* (A—C) after plasmorrhysis in  $\text{NaNO}_3$  (x—x),  $\text{KNO}_3$  (○---○), or  $\text{Ca}(\text{NO}_3)_2$  (○---○), moreover (D, E) on model droplets of glycerin (■.....■) and sodium stearate (△---△).

1) J. PEKAREK, *Protoplasma* 10, 510 (1930).



so I have only to give further particulars. In experiments with *plasmorrhized cells* of pericarps (*Taxus baccata*, *Physalis Alkekengi*), there is only a little agreement in the results using either sodium or potassium or employing calcium salts as plasmorrhyticis. It does appear that sodium and potassium ordinarily lower viscosity and calcium raises it. So in plasmorrhyticis with sodium or potassium the slope of the curve of viscosity rate (in percent of initial viscosity) plotted against the shearing stress decreases much quicker and stronger than the slope of the curve does if a calcium salt has been used. These differences, however, are less distinctly at other cells (leaf parenchyma of *Hyacinthus*), so that no general rule can be stated.

Taking in consideration the *temperature*, we find increasing fluidities, i.e. a decreasing slope of the curves of viscosity rates, between 26 and 31° just as between 10 and 15°C. These results correspondent with the course of some curves of anomalous behaviour of flowing speed referred to in the first part of this paper.

*Model substances* as water, glycerin, or sodium stearate show after staining with vital dyes a distinct difference between true fluids, such as water and glycerin, and elastic fluids, for instance sodium stearate. The flowing speed of the former substances is proportional to the shearing stress, and the calculated viscosity value remains nearly the same during the whole experiment. But if a solution of sodium stearate is used the viscosity values decrease more and more just the same as if protoplasm is sucked up in the capillary. Therefore, protoplasm as far as an elastic fluid of non-Newtonian nature is not obedient to the laws of NEWTON, POISEUILLE, and STOKES. This statement is as true as any that can be made of protoplasm.

### III. The anisotropy of the protoplasm within the capillary

The viscosity and in particular the anomalous behaviour of protoplasm against varied shearing stresses are part of the bigger problem of the *structural organization* of the material (WILLIAM SEIFRIZ). It is a colourless, viscous fluid with great light scattering power. In most cases the protoplasmic matrix of living cells exhibits no optical structure even under the highest powers of the microscope. Although protoplasm normally exhibits no optical structure, yet when *submitted to a stress along a definite axis*, a *fibrillar appearance* can sometimes be detected as O. BÜTSCHLI has already shown from homogenous protoplasm of *Gromia* caused to flow through the opening of the shell. Similar phenomena in *Amoeba* species and certain plant cells (G. W. SCARTH) indicate that the cytoplasmic particles can orientate themselves along definite axes when lying in a suitable field

of force, just as do those of albumen (W. B. HARDY). The existence of such orientations is not always obvious during life.

Under the ultramicroscope, as well as under the polarizing microscope, the protoplasmic fluid generally appears *optically void*. But elastic properties of colloidal fluids (sols) often are intimately associated with structure. There is a continuity in structure in these non-Newtonian systems which does not exist to the same degree in strictly Newtonian liquids. If an elastic solution is subjected to *shearing stresses* between two rotating cylinders, it shows a considerable amount of *double refraction*. This phenomenon is, commonly, the result of an orientation of optically anisotropic elements. It is well known that many substances become doubly refractive if they are deformed by external forces, an effect which is generally called *accidental double refraction*. The effect can be observed very easily, too, by an arrangement similar to the one used in the experiments as previously mentioned.

We use the deformation *apparatus* (fig. 1) consisting of capillary, water manometer and india-rubber aspirator bulb. Vertical to the axis of sucking up the protoplasm into the capillary, a beam of parallel, plane *polarized light* passes with its optic axis at a given radius through the flowing protoplasm. The light is produced by a powerful lower Volt lamp ("Monla" E. LEITZ) with adjustable collimator and iris diaphragm, collected by a condenser, polarized by a nicol prism and rendered nearly parallel by a second condenser. After having passed the capillary with the object and the space below the objective the light passes the microscope and the analysing nicol. The arrangement of the objective and ocular is rendered telecentric by two diaphragms of 0.15 mm. diameter. Thus only beams which run parallel or almost parallel to the axis of the microscope can enter the ocular.

If the protoplasm within the capillary is viewed as a whole between the crossed nicols a characteristic behaviour of the material appears while it is sucked up. At the first being directed without rules and regulations, the particles raise up vertically on the capillary wall and, finally, merge into each other in a homogenous layer. Now within the capillary the material forms a *liquid column* in which the optical axes of all the particles are upright radially.

The phenomena between crossed nicols come up to the assumption of structure. Therefore, if an angle of  $45^\circ$  is between the axis of the capillary and the principal cut of the nicols, a system of *many-coloured interference veins* presents in parallel with the capillary direction (fig. 4). If we permit the protoplasm to flow the interference strips remain the same as before, even at slight deflexions of the capillary. If, however, the flowing speed is growing more and more the particles are placed sloping and the interference veins disappear. Near the capillary axis the flowing speed is greater than near the wall, so the coloured strips are often not to see in the centre of the capillary.

Briefly considering how these facts are in relation to those from other investigations, one is reminded of H. PFEIFFER's experiments on the *disappearing of double refraction of myelin drops adhering* at walls of certain substances, and especially of G. VAN ITERSON's observations in experiments with the acid cadmium salt of the phosphor glycinic acid<sup>1)</sup>. This substance shows only a little rate of double refraction if it is sucked up below a cover-glass, but a much greater rate if investigated within a capillary. In case this happens *differentiated cross-bands of coloured interferences* arise within the mass.

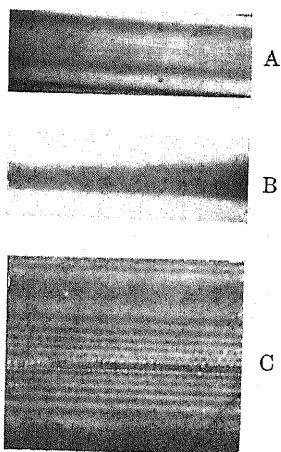


Fig. 4. The system of many-coloured interference veins in protoplasmic drops flowing within the capillary at middle (A), high (B), and very low flowing speeds (C) of the material (A and B  $^{300\times}$ , C  $^{650\times}$ ).

G. VAN ITERSON interprets the phenomenon as a spontaneous coagulation of the sol which is advanced by mechanical influences of the capillary wall. The question arises as to what extension his explanation is available for our experiments with flowing protoplasm. Perhaps the commonest conception is that the interference veins are caused by an *orientation of the protoplasmic particles* as described above. If this hypothesis is in the main correct, the broad centre band bordered with coloured strips is represented by a less orientation of the particles (molecules or micelles). Changes in the orientation and closeness of approximation of molecules are readily induced as many investigations have shown. Our experiments reveal that the cause of this phenomenon lays in the co-operation of *adhesion* at the wall and *flowing* through the capillary. Two possible explanations for the varying rate of double refraction and the behaviour of the interference veins may be mentioned. Either the phenomenon might be the super-position of a simple *saturation effect* and a linear term (*photoelastic effect*) increasing proportionally to the flowing velocity of the sucked up protoplasm, or the behaviour resembles to a certain extent in the *orientation of dipoles* (LANGEVIN function) in an electric field.

It can be said that a good agreement between theory and experiment can be obtained if it is assumed that the particles are optically and geometrically asymmetric. The prime factor, determining whether a fluid produces double refraction and interference bands during flow or not, is therefore the *anisotropy of the particles*. A certain amount of viscosity and strength of the shearing forces is in addition necessary, in order to overcome the disorienting tendency of the temperature motion.

Further complications with respect to the mechanism of double refraction and the breadth of coloured veins are encountered in the study of *plasmorrhized* states. It is apparent that these peculiarities are largely due to a variation of a physical property in the salt solutions which must be closely related to the influence upon the protoplasmic viscosity mentioned here above. As the same observations are to make by varying the *temperature*, at constant concentrations of the plasmorrhyticis, a similar conclusion is obtained.

1) H. PFEIFFER, Abh. Nat. Ver. Bremen 29, 185 (1935); G. VAN ITERSON, Proc. Akad. Amsterdam 37, 367 (1934).

In strongly acid and alkaline solutions the effect disappears as irreversibly, as under the influence of denaturing agents. In weak solutions of acids and bases and in a moderate range of *acidity* ( $p_H$  6.5 to 9.0) so far no striking changes have been observed. The results of varying the acidity are, however, scarce and have to be continued.

### Conclusions

In this brief survey of *three groups of experiments* I have confined myself to the most general results. In an account of the subject as given here it is not possible to avoid controversial points, and the outlook I have described may perhaps a personal one. On account of the doubly refractive behaviour of certain protoplasts certain authors think of the mesoplasm as based on a continuous structural *framework of a gel*, rather than upon a continuous aqueous phase like an emulsion or suspension (W. SEIFRIZ). But the rigid and elastic properties of protoplasm, too, are to explain on the assumption of its nature as a *non-Newtonian fluid*. It is shown, I hope, that it is possible:

- a) to bring about an *anomalous flow* of protoplasm within the capillary of the deformation apparatus, and to demonstrate an increasing shearing flow with increasing forces;
- b) to produce measurable *changes in fluidity* of protoplasts by means of varying shearing stresses;
- c) to indicate between crossed nicols an *anisotropic behaviour* of the protoplasm flowing through the capillary, i.e. an interference effect of coloured veins parallel with the capillary wall.

To summarize the *most fundamental results* of this paper we may say:

1. The protoplasm is not a pure liquid or a true solution but exhibits anomalous flow and is, therefore, not obedient to the laws of NEWTON, POISEUILLE, and STOKES.
2. As far as other fluids with anomalous flow, protoplasm behaves as if it contained aggregates of particles.
3. Under ordinary circumstances, an elastic fluid owes its anomalous flow, its changing in viscosity and its elasticity to the *presence of structural elements*.
4. Just so the described effect of double refraction or interference veins by means of flowing through a capillary is due to an optically and geometrically asymmetry of protoplasmic particles.
5. Under pressure the structure tends to broken down, and as the pressure is increased, there is an increase in fluidity or decrease in viscosity.

6. These structural features that gave to the flowing protoplasm its doubly refractive behaviour were probably responsible for its non-Newtonian character.

7. Protoplasm often behaves as though based upon the properties of a *non-Newtonian fluid*. Its tangential expansion  $\tau$ , as of any non-Newtonian fluid, is a function of the tangential shear  $\gamma$  and its change in time:  $\tau = f(\gamma \cdot d\gamma/dt)$ .

*Final remarks.*—In conclusion it gives me great pleasure to acknowledge my indebtedness to Professor E. G. BINGHAM (Easton, U. S. A.) for many helps and suggestions, and to Professor WILLIAM SEIFRIZ (Philadelphia, U.S.A.) for his great interest and kind criticisms during the development of these investigations. I also wish to express my best thanks to the optical enterprise E. BUSCH A.-G. (Rathenow, Germany) for the borrowing of several instruments especially in favour of the investigations of double refraction.

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## The Factors Locating the First Cleavage Plane in the Egg of *Chaetopterus*

By

**T. H. Morgan**

William G. Kerckhoff Biological Laboratories, Pasadena, Calif.

The original objective of the following experiments was to determine whether the first or second polar body (maturation) spindle, if held near the middle of the egg on the centrifuge, or if driven from the polar region into the clear zone of the egg by centrifuging, will bring about the division of the egg instead of returning to the surface and giving off a polar body, and also, to find out whether, if the polar spindles come to lie in elongated fragments of the egg, they will cause the fragments to divide rather than give off polar bodies on the surface of the fragment. The point at issue has some general interest as a cytological problem bearing on the nature of the mitotic figure that acts usually to give rise to the small polar bodies. The examination of these problems led to a study of the relation of the pole of the egg to the extrusion point of the polar bodies, and thence to the question of the presence of a dorso-ventral axis in the egg before its maturation. Since this last problem seems more important than those that were the initial starting points of the work it is the one especially treated here.

When the ripe eggs of *Chaetopterus* are taken from the parapodia the large germinal vesicle is present. In the course of fifteen minutes it breaks down, and the polar spindle develops, which then moves to the pole of the egg where it remains indefinitely if the egg is not fertilized. If the eggs are fertilized the first polar body appears in about 25 to 30 minutes and is followed in the course of 10 to 15 more minutes by the second polar body. At about 25 minutes later the egg becomes pointed at the pole, and then flattens after about seven minutes more when the antipolar lobe appears. The division of the egg starts five minutes later. These rates depend in part on temperature (between 23° and 25°C), and on the ripening of the egg.

In some of the earlier experiments the unfertilized eggs were centrifuged not earlier than fifteen minutes after removal to sea water, when the first polar spindle has developed. Large numbers of eggs in sea water were put into the centrifuge tubes. They form, after centrifuging, a compact mass at the bottom, many cells deep.

When broken apart by proper centrifuging the pieces, large tops (with or without oil caps), middles and bottoms (Figs. 1-12) remain at the outer end of the tube, but under certain conditions tiny oil drops come off which rise in the water of the tube. These tiny oil fragments float in the water, and, when removed to dishes of sea water, collect on the surface. Each consists of oil and a little clear protoplasm. They do not, as a rule, contain all of the oil of the egg, but only part of it. They are so small that unless the tube is examined with a lens they may easily be overlooked.

In order to avoid crowding of the eggs, fewer of them were put into the tubes over a solution of gum arabic in sea water. The eggs of *Chaetopterus* are easily stratified, but not elongated or broken apart on the gum, even at a much higher speed of centrifuging than suffices to break them apart in sea water. In other words the membrane does not break down when the eggs lie on the gum, and its presence prevents the egg from elongating (or allows only slight elongation).

To obtain satisfactory fragments of the eggs in sea water it is best to start with a slow rate (1850 r.p.m.). The outer end of the centrifuge tube was about 19 centimeters from the axis. At this rate the eggs are stratified in five minutes. The speed is then increased (2970 r.p.m.) for several minutes (10 minutes) which elongates the egg, and then for about two minutes a higher rate (4530 r.p.m.) is used, which breaks the membrane, usually at the yolk part. The yolk pinches off from the rest of the egg, and the top often remains inside the broken membrane. The constrictions that separate the egg into fragments may take place at different levels, depending partly on the time and rate of centrifuging, and also on where the membrane breaks to let the yolk squeeze out. The elongated tops and middles, or even whole eggs that have not broken, round up in the course of a few minutes after removal from the tube.

The problem of the orientation of the eggs in the centrifuge tube brings up several interesting questions. When stratified over gum after the polar spindle has formed (15 minutes) and then fertilized, it is found that the first polar body comes off in nearly all eggs at the side of the clear protoplasmic zone, sometimes even near the equator of the egg. It may appear that the spindle can not pass through the oil cap, assuming this to lie at the pole, and turns to one side. If this were the explanation one would expect the polar body to appear at least on one side of the oil or at its edge; but, while this does occur, it is not the rule. The preceding interpretation rests however on the assumption that the eggs orient on the centrifuge with the true pole turned centripetally, and consequently with the oil cap



at that pole. On the other hand, even if the eggs should fall, when centrifuged in water alone, with the pole toward the axis of the centrifuge they might, when they become packed together at the outer end of the tube, be turned in all possible positions. This would mean random stratification, and if this occurs the stratification should bear no relation to the polar axis of the eggs. There is, however, evidence to the contrary even although it be granted that the crowding may affect the orientation to some extent. For example: if eggs are fertilized before centrifuging and are then centrifuged at a moderate rate, so that they are not elongated, at the time when the first polar body is already extruded, it is found that it lies at one side of the clear zone in most of the eggs, and not in the center of the oil cap. In fact, it is in approximately the same position as when the unfertilized egg is centrifuged and the polar bodies come off afterwards. Furthermore, the centrifuged eggs become pointed later exactly where the polar bodies lie (now two of them), and shortly after this the yolk lobe appears opposite the polar bodies, rather than opposite the oil cap. This can only mean that the eggs orient themselves on the machine with the true pole at one side, for the polar bodies, if extruded before centrifuging, certainly lie at the true pole of the egg. It would seem to follow that the top of the egg, i.e. its uppermost point in response to gravity, is at the surface immediately above the large germinal vesicle, hence the egg turns on the machine with its lightest region towards the center of rotation and with the true pole at one side. In other words the oil is not driven to the true pole, but to one side of it.

In order to find out to what extent the crowding of the eggs at the end of the centrifuge tube may interfere with their orientation some eggs were centrifuged over thick gum arabic dissolved in sea water. In this case fewer eggs were centrifuged, only enough to give a single layer on the gum. In the first test, eggs that had been in sea water for 15 minutes were centrifuged over gum at 1850 r.p.m. for 5 minutes which suffices to stratify them. The spindle is at this time beneath the true pole of the egg. When removed and examined under the microscope some of the eggs show by the clearer region where the polar spindle lies. This is usually at one side of the oil or not in the oil field at all. Most eggs show the oil field intact. A count of eggs showed 15 with the polar spindle excentric in the oil field, one or two at or near the middle, and 45 with an intact field i.e. only one fourth of the eggs showed the true pole to lie at or near the oil field, and in nearly all cases at one side of or outside the oil field. The spindle in other eggs was either in the clear zone or beneath the oil. When fertilized the polar bodies came off corresponding to



the above interpretation, i.e. most of them at one side of the clear zone.

Another lot of the same unfertilized eggs, that had stood in sea water for 35 minutes, were similarly centrifuged. The longer interval may be supposed to have given the polar spindle more time to approach near the pole. The results were much the same as in the last case, namely, 26 spindles were in or at the edge of the oil field, while 77 eggs showed no clear region or crescent in the oil.

A third lot of the same eggs were fertilized. After 36 minutes the second polar body was out. The eggs were then centrifuged for 10 minutes at 1850 r.p.m. Owing to the longer centrifuging the oil field was a little more concentrated. No polar bodies were to be seen in the middle of the oil field. They lay at the side of the oil or in the clear zone.

Finally unfertilized eggs that had stood for two hours in sea water were centrifuged over gum in the two tubes of the machine at 1850 r.p.m. for two minutes. One lot (A) was then taken out and examined. The oil field was small. Only a few eggs out of 50 counted, showed a clear spot at the edge of the oil, and only two out of 50, in the middle of the oil field. The eggs in the other tube (B) were centrifuged further for 5 minutes at 2970 r.p.m. When removed no clear spot was found in any of the oil fields. If in some eggs the spindle lay at the center of the oil it must have been driven below it. Spindles that lay near the edge of the oil might not now appear in the smaller oil field, others may have been driven below the surface, but there is not much evidence that such was the case.

The following observations illustrate what happens when eggs are centrifuged at once in sea water, i.e. before the polar spindle is formed and during that time. Eggs were taken from the parapodia, washed quickly in sea water, placed on the centrifuge and rotated for 5 minutes at 1850 r.p.m. They became stratified, but the three layers were not as sharply separated as when centrifuged later or at a higher speed; the oil field for example is not so deep and covers more of the surface. Removed to a dish of sea water some eggs lie at first on the side; others with the oil uppermost. At this time the polar spindle has not come to the surface. Fourteen minutes after removal, the oil field turned up in many eggs, it lay obliquely in others, and to the side in the rest. Only a few eggs show a clear spot in the center of the oil which, in such cases, forms a ring around the clear spot. Four minutes later the eggs were fertilized. Eleven minutes later the first polar body is coming off. At this time many eggs lie on the side and the polar body is coming off at the side i.e. in the clear zone; in others the oil field is crescentic or notched indicating that the polar body is coming off in or near the oil field,

which itself may now be shifted to one side of its original position. Only a few eggs have a clear spot at or near the center of the oil field. When the second polar body comes off beneath the first one, the first polar body can be seen more clearly. The oil field is uppermost in many of the eggs. In about 30% (estimated) there is a clear spot in or at the edge of the oil. In those eggs in which the oil field is intact the polar bodies lie in the clear zone, sometimes near the oil, sometimes in the middle of the clear zone, and in extreme cases at the horizon, i.e. nearly  $45^\circ$  away from the center of the oil.

In another similar experiment the eggs were put in the centrifuge at once and rotated a little longer (7 minutes) at a slightly higher rate (2700 r.p.m.). When removed the eggs were somewhat elongated or pear shaped. The oil field was more concentrated (smaller in circumference). Eleven minutes later most of the eggs were spherical or nearly so. Very few showed a clear spot at the edge of the oil field. The eggs were fertilized 2 minutes later. Eight minutes later still some of the eggs showed a clear spot notching the edge of the oil field (in the ratio of 32 with a notch to 100 without a notch); rarely was there a clear spot in the middle of the oil field. In some eggs it was obvious that the oil field was being pushed to one side as the spindle came to the surface, approaching the yolk more on one side. When the polar bodies appeared they lay, in most of the eggs, at the edge of the clear zone. Later when the eggs became pointed, the polar bodies were at the pointed end, showing that they had come off at the true pole. A third experiment of the same kind need not be recorded as the results were the same.

Since the eggs centrifuged in sea water tend to become elongated (although they soon round up) eggs were next centrifuged over gum arabic where they retain their spherical form.

Eggs in sea water were placed over gum in the centrifuge tubes immediately, and allowed to settle. They were then centrifuged for 5 minutes at 1850 r.p.m. When removed most eggs came to lie with the oil up, the rest on their sides. Seven minutes later, all had turned up; only six eggs in a hundred had a clear spot in the center of the oil. Two minutes later a larger number showed a crescent shaped oil field. Eleven minutes later 9 had a crescentic oil field and 76 not, only two showed the clear spot in the center of the oil field. After fertilization the oil field lay in most eggs obliquely with respect to the yolk, i.e. it had been pushed to one side. The polar bodies when seen (outside the oil field) lay at the edge of the clear zone. Some of the same eggs were kept for 17 minutes on the machine at the same speed. During this time the polar spindle must have developed. These eggs were well stratified but not elongated to any extent. In

sea water some of the eggs (54%) turned the oil field up, others lay on the side. Rarely was the oil field dented. Twelve minutes later nearly all the eggs lay obliquely on the side. After fertilization the eggs lay with the small oil field up or a little obliquely; only 7 eggs had a clear area at the edge of the oil field and 50 not. Later the polar bodies lay at the side of the clear zone, none showed a clear center in the oil, which had now spread somewhat.

These results with gum arabic are practically the same as those in sea water, showing that slight elongation of the egg in the latter had not affected the results. It is clear, therefore, that the top of the unfertilized egg, which is its uppermost side in response to gravity, corresponds to the oil field, and that the true pole lies excentrically with respect to the lightest region of the egg, both before and after the polar spindle has moved toward the surface of the true pole.

It has been pointed out that when the eggs are taken from the parapodia, washed in sea water and allowed to settle on the bottom of a flat dish, under the microscope they are all apparently alike, i.e. with the germinal vesicle turned exactly upward. As the germinal vesicle (egg nucleus) disappears and the spindle develops, a clear region appears at one side of the egg, which, as subsequent observation shows, is the true polar field of the egg. In many of the eggs it lies almost at the equator, in others it lies somewhere between the original top of the egg and the equator, i.e. in an oblique position. Two interpretations seem possible: (1) The eggs turn to one side as the spindle develops so that the original top of the egg comes to lie at one side; (2) the top of the egg is not the true pole, that lies to one side. The polar spindle moves towards the true pole. To settle this point, eggs resting quietly on the flat bottom of the dish in a single layer were carefully watched during the time when this change takes place.

A group of eggs that had been allowed to settle was sketched and watched for any evidence of turning to one side as the change described above takes place. No evidence was found that the eggs shift as a whole to one side. A few eggs in each group can be found that are not quite spherical and these furnish a better chance to observe any turning, especially eggs that are a little elongated. No turning was observed in them. Sometimes such eggs developed a clearer region at one end of the elongated egg, and here any movement as a whole would be easily observed. This evidence in itself might not be regarded as entirely convincing were it not confirmed by other observations. For instance: if the eggs are slowly centrifuged over gum after the first or both polar bodies are extruded, it is found in most cases, as described above, that the polar bodies do

not lie at the center of the oil, but to one side of it, or even entirely outside of the oil field. This is consistent with the view that the original part of the egg that turned uppermost in response to gravity is still the lighter part of the egg. The true pole, where the polar bodies lie, is still at one side of this region. If the eggs had turned as a whole this would not have been the case. The conclusion is obvious, namely, that the germinal vesicle does not lie beneath the true pole, and the lightest part of the egg is not at the pole, nor is it there after the maturation spindle has moved to the true pole. It follows either that the germinal vesicle may be anywhere in the polar hemisphere with respect to the pole, or that it lies excentric to the pole but in a definite position in the egg.

Two sets of eggs were fertilized 15 minutes after removal and allowed to settle. In each set about 25 eggs were drawn with the camera, viz. those eggs that showed distinctly the clear area at the side. A mark was placed on that side of the drawing. The eggs were closely watched until the first cleavage appeared. In the first dish the first cleavage plane came in vertically, i.e. through the pole and the uppermost part of the egg. In three eggs the plane was oblique. In the rest of the dish, where the eggs were not drawn, there were counted 240 eggs with the first cleavage plane vertical and only 16 in which it was oblique. In a second dish the first cleavage was vertical in 12 eggs and oblique in 3 eggs. Two eggs divided into 3 cells.

In order to meet the possible criticism that the position of these eggs had been affected by the entrance of the spermatozoa, another lot of eggs was allowed to settle as soon as removed. After 25 minutes, concentrated sperm on a glass rod was gently added to the top of the water. A group of eggs was watched at the time, and it was observed that their positions did not change. Here 20 eggs divided vertically, one obliquely, and two into 4 cells. In two cases the vertical furrow was at right angles to the presumptive location of the pole which may actually be the case, unless the pole had been wrongly identified.

In a third experiment the eggs were fertilized as soon as removed, i.e. before the germinal vesicle broke down. After they had settled and the true pole indicated, the eggs, as before, were drawn. Here also the first furrow came in vertically and through the pole in 20 eggs; in two cases the furrow came in nearly at right angles to the marker; one egg divided into 4 cells; and in one the lobe came in on the top of the egg. The last must have been an egg turned upside down.

These results demonstrate that the top of the egg lies in the

plane of the first cleavage, and since it was in this region that the egg nucleus originally lay, it follows that its excentric position occupies a definite position in the egg.

Now since it is known that the second cleavage of the eggs of annelids is in or near the plane of bilateral symmetry, and since the second plane comes in at right angles to the first, it follows that the germinal vesicle lies in the dorso-ventral axis of the egg. It follows that the egg of *Chaetopterus* has its dorso-ventral relation determined both before maturation and before fertilization. The interpretation of this relation will be discussed later.

### Whole Eggs Kept on the Centrifuge During the Time of Formation of the Maturation Spindles

In *Ilyanassa*, the so-called "giant polar body" is formed only when the eggs have been elongated on the machine and when they still retain this shape during the pinching apart of the two moieties. In *Crepidula* there is no evidence to show whether those eggs that divide to form giant polar bodies were elongated before or during the division. Both in *Ilyanassa* and *Crepidula*, a spermatozoon has already entered the egg when laid, while in *Chaetopterus* the polar spindle does not divide unless the egg (or the fragment) is fertilized. Hence for comparison the eggs of *Chaetopterus* must be fertilized before centrifuging if the polar spindle is to proceed in its division.

Eggs were kept in sea water for 11 minutes, then fertilized. Seven minutes later the eggs were put on the centrifuge over gum and rotated at 1850 r.p.m. for 10 minutes, then at 2970 r.p.m. for 10 minutes, during which time the first polar spindle has divided in the normal egg. When removed a polar body was seen to come off in 13 minutes in a few eggs; at the side of the egg or at the edge of the oil field (the first polar body was out in the control at this time). Later some of these centrifuged eggs were seen to have two polar bodies at the side.

Some of the same eggs (in the sister tubes) were centrifuged 10 minutes longer than the last (at 2970 r.p.m.). Some of the eggs were slightly elongated. Most or nearly all of them failed to give off polar bodies, and 48 minutes later some of them divided (unequally); some had an antipolar lobe, other had not developed a lobe at this time. Many eggs had not divided. These were stained and found to have their chromosomes deep in the egg, i.e. in the protoplasmic zone.

A third repetition gave similar results. The eggs were kept in sea water for 11 minutes, then fertilized. Seven minutes later they were put into the centrifuge and rotated for 2 minutes at 1850 r.p.m.,

then for 5 minutes at 2970 r.p.m., then for 10 minutes at 3700 r.p.m. No clear areas were present in the oil fields. After 19 minutes a polar body was seen in one egg at the edge; none in the others. Later a few more showed polar bodies at the side of the clear zone. Thirty seven minutes after removal some eggs (about 20%) showed lobes developing or were about to divide. Ten minutes later more were dividing, some with and some without a lobe. In both cases the division was somewhat unequal, but sometimes equal. Stained preparations confirmed the observations showing that some of the eggs divided without extruding polar bodies. They are probably pentaploid. Some of the undivided eggs showed chromosome plates in the interior of the egg.

Another experiment was made in an attempt to separate the daughter halves of the first polar mitosis. After eggs had stood for 15 minutes in sea water, they were separated into six lots, fertilized successively at intervals of one minute. Then after 17 minutes they were mixed and put into the two tubes of the centrifuge over gum, and centrifuged at 1850 r.p.m. for 3 minutes, then at 2970 r.p.m. for 10 minutes. When the eggs were put on the centrifuge, the second lot had the first polar body just appearing. During the 13 minutes on the centrifuge the first polar division was due. Hence there was a chance of catching some eggs in which the outer group of chromosomes of the first polar spindle was attached to the surface and the inner group free to move into the clear zone of the egg. Should the separation of the two groups lead to a division after removal this would have been observable. No division appeared. Later the eggs divided into two unequal parts as does the normal egg. The division was 13 minutes later than that of the control. Since the polar body was not observed in most eggs after staining, it is evident that it must have been held below the surface in most eggs and may have divided there without causing division of the egg.

Some remaining eggs of the same six lots were put into the two centrifuge tubes at 36 minutes after fertilization. At this time the second polar body was out or coming out in some of the eggs. The eggs were kept on the machine for 15 minutes. Three minutes after removal one lot became irregularly lobed, but few were lobed in the other lot in which the eggs remained spherical. The former became spherical again after 12 minutes. The cleavage was delayed, but most eggs went into 2 and 4 cells, some of them not quite regularly. No division before cleavage was observed. The stained preparations of the first tube showed the first polar body out in some eggs and chromosomes in the clear zone. In other eggs one polar body was out and the chromosomes inside. In the second tube one or possibly two polar

bodies were out and the chromosomes in the clear zone. Nearly all of the first set divided into two unequal cells with yolk lobes.

Two other experiments of the same set gave similar results, but as the eggs were not in first rate condition the results need not be reported. In neither set did division occur before the cleavage appeared.

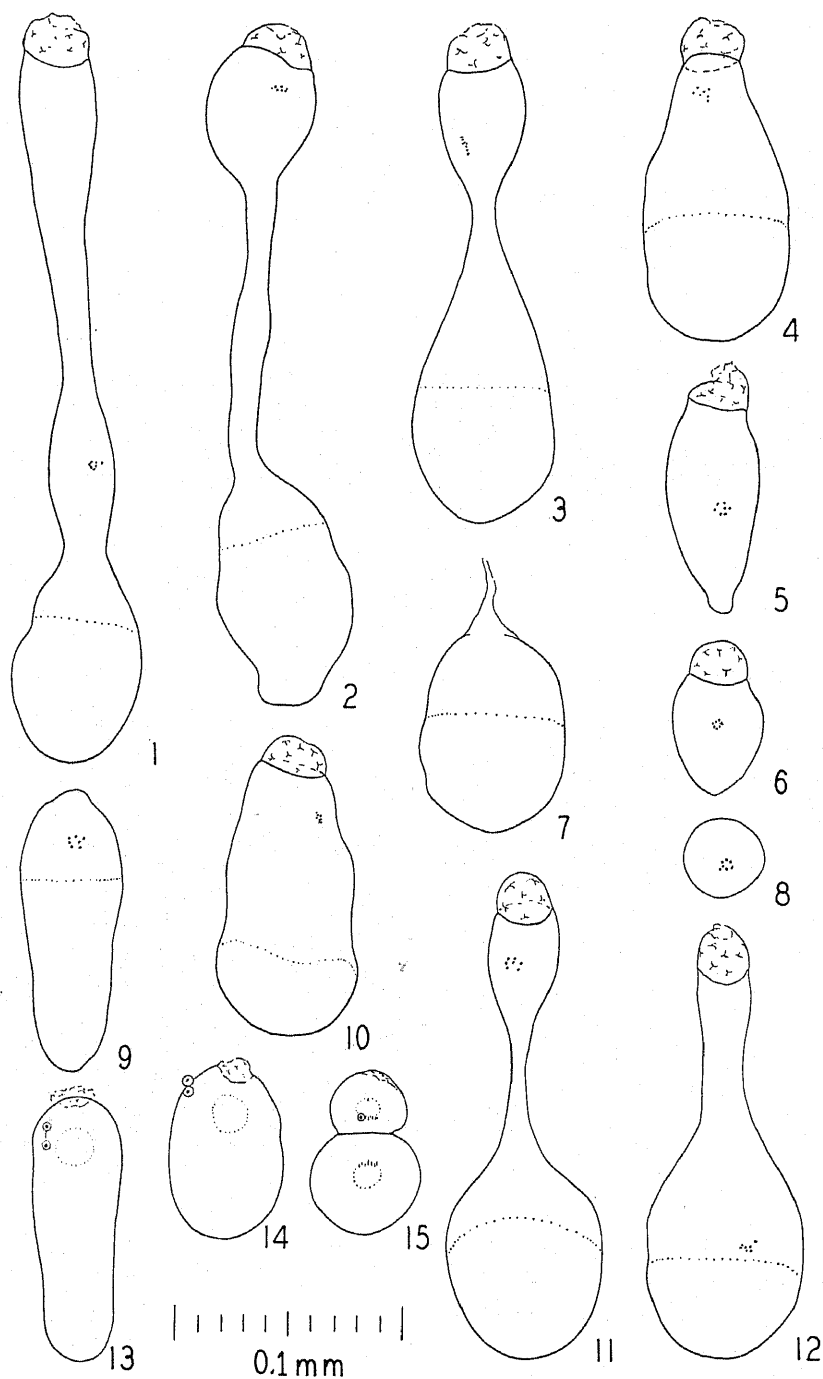
Two comparisons may be made. The *Chaetopterus* eggs orient in such a way that the polar spindle is not exactly at the centripetal pole of the egg but at one side, hence there may be less chance of centrifuging the protoplasm away from the polar hemisphere as can be done in the oblique or inverted eggs of *Crepidula*. Consequently there may seem to be less likelihood of separating the two anaphase plates widely. Whether this has anything to do with the failure of the maturation spindles to divide the egg can not be stated. Secondly, the eggs centrifuged over gum are not elongated, hence the situation is different from that of *Ilyanassa*.

Summing up it may be said that by preventing the polar bodies from coming off by keeping the eggs on the centrifuge, the polar mitotic spindle does not bring about a division of the egg. There was no evidence pro or con that the centrifuging caused the daughter halves of the anaphase polar nuclei to separate. In *Crepidula* where Conklin accomplished this, it is explained as due to one daughter group being held at the surface while the other daughter group is dislodged, owing to the cytoplasm being carried into the egg (or possibly as I have suggested along one side). This abnormal condition, he thinks, is responsible for the subsequent division of the egg. On the other hand in *Ilyanassa* according to Clement and Morgan the two daughter groups of chromosomes of the second spindle move apart only *after* the spindle has been driven as a whole or moved into the constricted region of the egg. The daughter nuclei move or are carried apart there and the protoplasm constriction may sometimes separate them.

#### Fragments of Eggs Centrifuged in Sea Water and over Raffinose

When eggs are centrifuged in sea water at 3700 to 4230 r.p.m. they form a compact mass in the end of the tube. When released they are found to be broken up into fragments of different sizes. Some of these are tops with or without an oil cap, some are middles without oil or yolk, and some are bottoms consisting mainly of yolk (Figs. 1-15). It appears that in sea water the tough membrane of the egg breaks most commonly at the yolk end of the egg, the yolk





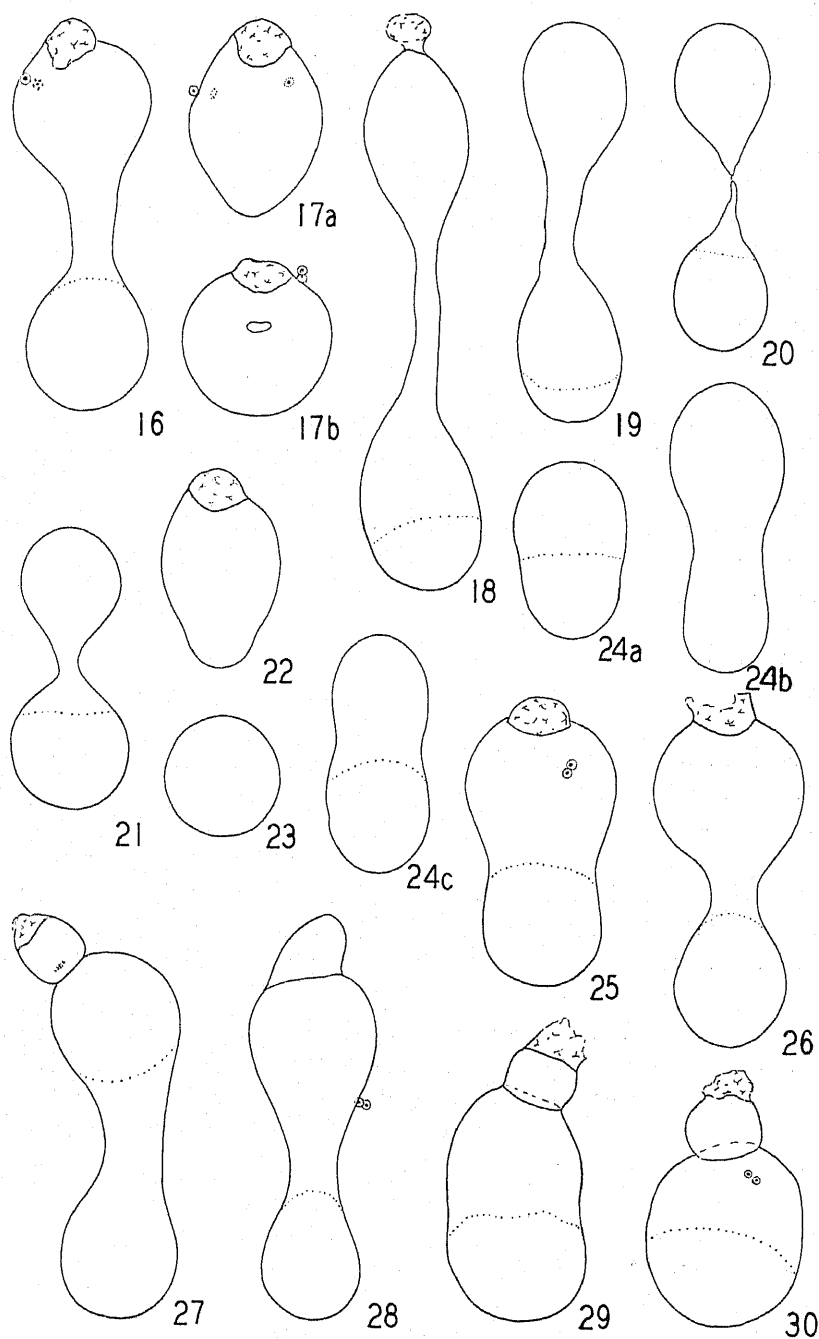
Figs. 1-15. Eggs centrifuged in sea water (see text) that have been elongated, or pinched apart, into tops and bottoms. These eggs were killed in Kleinenberg's picrosulphuric, and stained in Delafield's haematoxylin.



squeezes out and is pinched off, or breaks off when the eggs are removed from the tube. The oil may be in part or entirely pinched off. Although many fragments may be obtained in this way, they vary so much in size and in other differences that it is difficult to make a statement as to their behavior without going into some detail.

Twelve different lots of eggs were studied: some of them were centrifuged before and during the time of the first polar body; others during the time of the second polar body, and others were kept on the machine during the time of extrusion of both polar bodies in the control. In some cases one, in others two polar bodies were given off from the tops or middles. In other cases no free polar body was observed. Later, at the time of the normal cleavage, many of these fragments divided into two unequal parts as in normal cleavage. In none of the cases was there any evidence that either the first or the second polar spindle divided the fragment, even when, as in some cases, the fragment was kept elongated on the machine when the mitoses were due. Preserved eggs show a group of chromosomes somewhere in the clear, now stained zone (Figs. 1-12). Sometimes the chromosomes are in the globular top that is about to be pinched off or has been pinched off (Figs. 2-6); at other times the chromosomes may be in the constricted zone (Fig. 1). If the eggs have been on the centrifuge during the time when the polar bodies are due these may be given off at the side of the stained zone. In most cases the group of chromosomes lies near the surface rather than in the interior of the stained zone. Consequently polar bodies are given off and a division of the egg or fragment does not take place.

After the preceeding experiments had been finished, and only a few days remained to complete the work, a new method was found that is for most purposes greatly superior to centrifuging in water to break the eggs apart, or over gum to hold them intact. A saturated solution of raffinose in sea water was made. A little of this (about 5 drops) was put into the bottom of a centrifuge tube. Eggs in sea water were added on the surface of the raffinose, mixing as little as possible. Rotated on the centrifuge the eggs lie on the surface of the raffinose in a single layer. No crowding takes place and better orientation is secured. After centrifuging, the eggs form a raft floating on the raffinose, and can be removed by a pipette with very little of the sugar. The extent to which they may be elongated or broken apart is easily regulated by the rate and time of rotation. The method, so far as obtaining uniform tops for subsequent study of cleavage, is immensely superior to sea water alone. I have, however, not attempted to follow this up except to record a few general observa-



Figs. 16-30. Eggs centrifuged over raffinose. The elongation of the egg is shown in some of the figures; in others, tops, middles, and bottoms have pinched apart on the machine or on removal. All from one set; 16 to 24c killed at time of removal from machine. 25 to 30 twenty three minutes later.

tions on the first and second divisions. The following eight experiments record the results with raffinose carried out to study the behavior of the polar spindles.

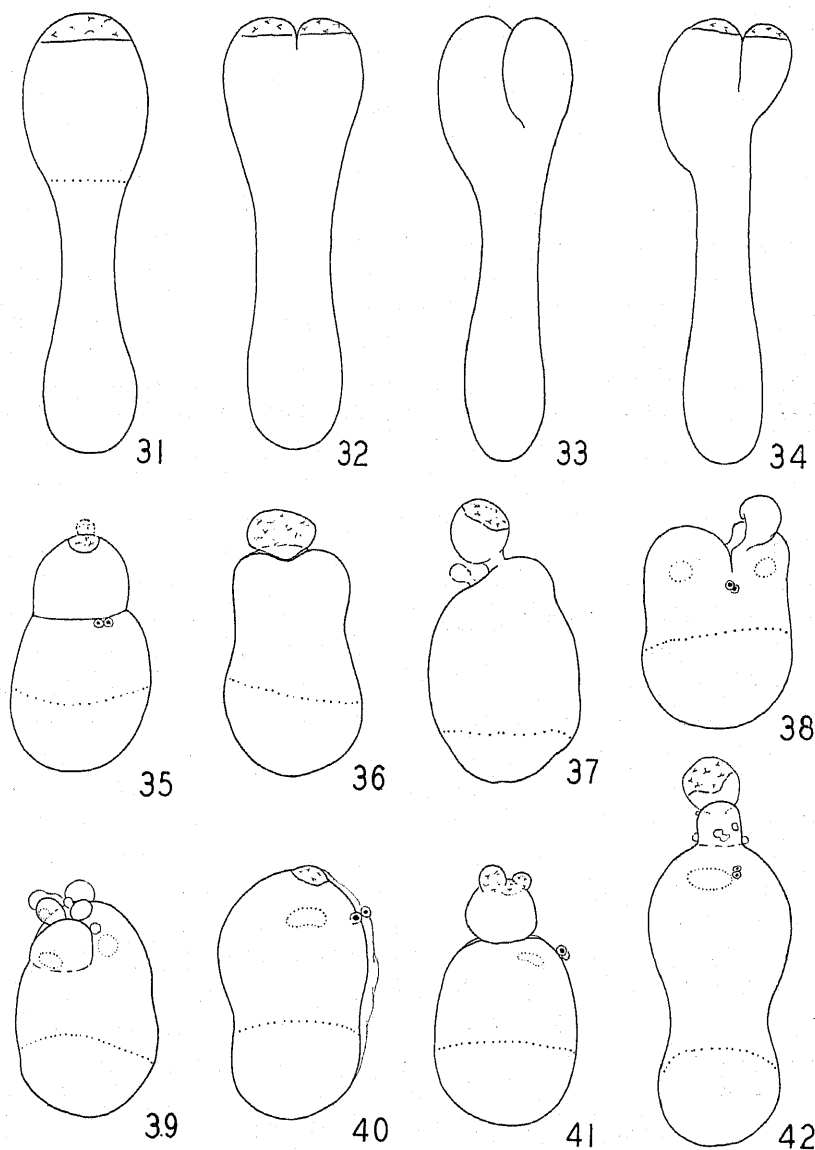
Eggs were allowed to stand for 10 minutes and were then fertilized. After another 10 minutes they were centrifuged over raffinose for 3 minutes at 1850 r.p.m., for 5 minutes at 2970 r.p.m., then for 5 minutes at 3700 r.p.m. They were elongated about 3 times their breadth, and most of them were dumb-bell shape. Some tops had pinched off. Two polar bodies were found in some eggs and tops. Fifteen minutes later many of the eggs were spherical, but none had divided. One hour and nine minutes after fertilization the whole eggs began to divide with lobes.

Some of the above eggs were left in the centrifuge and rotated at 2970 r.p.m. for a time not recorded, probably 13 to 15 minutes. None had divided when taken off. Most were dumb-bell shape (Fig. 16). Some tops had pinched off (Figs. 17 a, b). Two polar bodies were found in some of the stained eggs. Twenty two minutes later some of the tops began to divide into two unequal cells without lobes. In these two experiments the polar spindles did not divide the eggs or the tops, although the eggs were elongated during the time when the polar division was due.

Eggs that had stood 13 minutes were fertilized. They were centrifuged over raffinose 10 minutes, later for 3 minutes at 1850 r.p.m., for 5 minutes at 2970 r.p.m., for 10 minutes at 4230 r.p.m., and then for 8 minutes at 2970 r.p.m. again. Most of the eggs were elongated, and were several times as long as broad, and in many of them some or all of the oil was lost (Figs. 18-20), others were dumb-bell shape, others had separated into tops and bottoms (Figs. 20-24c). Most of the tops cleaved 30 minutes later, when the controls showed lobes, into two unequal parts without lobes. In the poorly stained preparations the nuclei and polar bodies did not show.

Other eggs of the same lot were centrifuged for 5 minutes after the second polar body appeared, for 4 minutes at 1850 r.p.m., for 5 minutes at 2970 r.p.m., for 5 minutes at 4230 r.p.m., and for 2 minutes at 2970 r.p.m. again. The eggs were about three or more times as long as broad, and dumb-bell shape (Fig. 31). They shortened, as usual, in the picro-sulphuric (Figs. 25-30). Some of the oil was lost in some of the eggs. A few of the eggs showed a constricted protoplasmic sphere below the oil (Figs. 27-30). This, and another case, are the only ones that might be interpreted as forming a giant polar body. They had been on the machine for 16 minutes during which time the second polar mitosis took place in the controls. But this interpretation is doubtful, because in some of these eggs, as the figures

show, two small polar bodies are present. Also, with one possible exception (Fig. 27), dividing nuclei were not present in the constriction, and in none of them was there any nucleus visible in the small ball. Four minutes later the tops showed a partial division

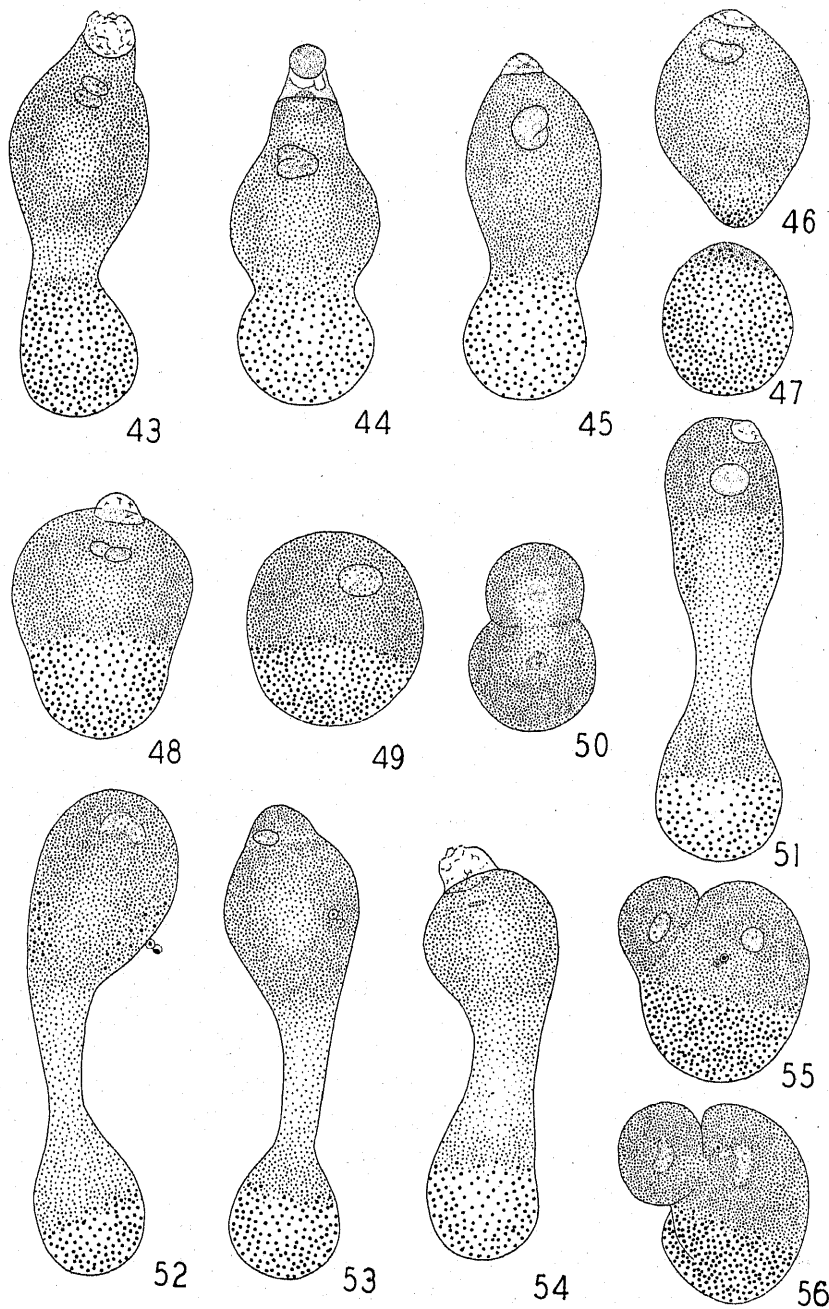


Figs. 31-42. Eggs centrifuged over raffinose. 31 to 34 drawn from living eggs. The first one immediately after removal from machine; the other three were drawn free hand a few minutes later when the first cleavage has begun through the oil end of the elongated egg. 35 to 39 from the same set, nine minutes after removal (preserved and stained eggs) when the first cleavage is taking place. 40 to 42 eggs of another set ready for cleavage.

through the oil field (Figs. 32-34) and in some of them a small cell was cut off at one side. This was two or more minutes before cleavage in the controls took place, but is probably nevertheless a cleavage division. Some of the eggs that had been left on the raffinose in the tube were put back on the centrifuge for 6 minutes more which kept them elongated. Fourteen minutes after removal a few showed a notch in the apex which was a cleavage division. In addition to the vertical cleavage division, that was appearing at this time, a few eggs pinched off the oil that lay in the cleavage plane. In some of these eggs, with little oil, some of the protoplasm was pinched off with the oil (Fig. 37), other eggs gave off several droplets that are obviously not normal occurrences (Fig. 39). It seems highly improbable that these formations, occurring at the time of cleavage, had anything to do with polar body divisions.

In the next set eggs were fertilized 14 minutes after removal. Four minutes after the first polar body had appeared the eggs were centrifuged for 5 minutes at 1850 r.p.m., for 10 minutes at 2970 r.p.m., and then for 5 minutes at 3700 r.p.m. When removed they were about three or more times as long as broad. The preserved eggs showed a large nucleus near the stained end in most eggs (Fig. 40). Rarely an egg was found with a small ball constricted off at the polar end (Figs. 41, 42). These balls resemble those in the last set, but since two polar bodies were present at the side of the stained zone it is improbable that they are to be interpreted as polar bodies. Possibly the membrane broke at the top setting free the oil and a bit of the protoplasm beneath it, which later constricted off. The oil was absent in some eggs. The eggs were spherical 13 minutes later and two large nuclei were visible in the stained egg. Twelve minutes later most were dividing into two unequal cells. The controls were about to divide at this time.

Eggs that had stood for 13 minutes were fertilized. Twenty minutes later, when the first polar body was just out, the eggs were centrifuged over raffinose for 3 minutes at 1850 r.p.m., for 3 minutes at 2970 r.p.m., and 3 minutes at 4230 r.p.m. Since the eggs were still spherical they were centrifuged for 6 minutes more at 3700 r.p.m. The unbroken eggs were then five or six times as long as broad, and had lost some of the oil. They shortened in the preservative (Figs. 43-45). Some tops had come off (Fig. 46) that were larger than the bottoms (Fig. 47). Seven minutes later the eggs had contracted to half their former length, and were spherical six minutes later (Fig. 49). The tops (with oil and without yolk) divided into two unequal cells without a lobe (Fig. 50). In this set again none of the tops were divided by the polar spindle. The second polar body



Figs. 43-56. Eggs centrifuged over raffinose. 43 to 48 elongated eggs, and a top and bottom; killed on removal from machine, and stained. 49 and 50 later stages of same. 51 to 54 eggs of another lot, killed on removal from machine. 55 and 56 thirteen minutes later, dividing into a small and a large cell.

may have been given off before the eggs were elongated by the last centrifuging, but it was not found in the eggs after centrifuging. As the figures show, a large nucleus was present when the eggs were removed from the machine (Figs. 43-48). This nucleus is often double or lobed and represents no doubt the combination of the sperm and egg nucleus. The latter may represent, in some cases at least, all the chromosomes of the second polar body plus those of the sperm nucleus, and possibly, in some cases at least, all the chromosomes of both polar bodies when the first polar body failed to come off. It is difficult to detect the polar bodies in this set of centrifuged eggs. They may have been driven off the surface of the egg when it breaks through the membrane and elongates, or been flattened against the egg.

Eggs that had stood for 9 minutes were fertilized, and 25 minutes later, when the first polar body had just appeared, the eggs were centrifuged over raffinose for 2 minutes at 1850 r.p.m., for 2 minutes at 2970 r.p.m., and 5 minutes at 3700 r.p.m. The eggs were about twice as long as broad when taken off, and were not broken apart. Chromosomes could not be detected in the stained eggs at this time, and there is no evidence of a second polar spindle. Nine minutes later the eggs were nearly spherical, and the stained eggs showed two large nuclei in the middle of the stained zone. Lobes appeared later on those eggs that were about to divide. The eggs had not been fragmented on the machine.

After standing for 10 minutes the eggs were fertilized. Two minutes after the first polar body appeared the eggs were centrifuged over raffinose for 5 minutes at 1850 r.p.m., for 10 minutes at 2970 r.p.m. and for 5 minutes at 3700 r.p.m. The eggs were much elongated and dumb-bell shape as shown in Figs. 51-54. When removed they were even more elongated than the figures show, for here, as in all cases, the eggs had shortened immediately when put into the picro-sulphuric preservative. In most of these eggs a large nucleus is present in the stained protoplasm which represents the segmentation nucleus. Two polar bodies were detected, which means that the second polar body had been given off at the side of the clear zone in some eggs at least. A partial oil cap was present on some eggs, but all the oil had been driven off in others. The eggs became shorter, and after 11 minutes were notched at the polar end. Eight minutes later they were dividing irregularly into two cells that were very unequal (Figs. 55, 56). One egg had divided into nearly equal parts. The control eggs were divided at this time. Here again the polar spindles failed to divide the greatly elongated eggs that had been on the machine when the second maturation division took place.



### The Stratification of the Eggs in Relation to the Polar Axis

In 1906 and 1909 F. R. Lillie reported a series of centrifuging experiments with whole eggs of *Chaetopterus*. His results indicated that some of the eggs at least are stratified at different angles with respect to the primary axis. This would mean that the eggs (in water) are not all oriented in the same way on the machine. He thought that while most of the eggs, after maturation, orient with the heavier yolk hemisphere centripetally, "various causes combine to overcome this". The crowding of the eggs in the outer end of the tube would be one of the agents of this sort. In Wilson's earlier paper (1929) he did not discuss the question of orientation on the machine, the implication being that the hyaline fragments whose cleavage he described may represent any part of the original egg. In his second paper (1930) he examined the question of orientation and concluded that there is a "very marked tendency" for the eggs to orient themselves. He centrifuged eggs, that had extruded the first polar body and found that 70% showed the polar body in the oil cap, and 9% just outside. After the extrusion of both polar bodies, 70% of the eggs showed the polar bodies under or just outside the oil cap. These figures differ a good deal from those that I have here recorded, but are not essentially different since I was mainly concerned in finding cases where the polar spindle lay exactly in the center of the oil cap, at its edge, or outside of it. Moreover eggs centrifuged in sea water if crowded would be expected to give a somewhat different result from those over gum, since "various causes" may here affect the orientation. The area of the oil field is also a factor. The fact that the lightest region of the egg does not coincide with the true pole either before or after maturation was unknown to both experimenters.

The direction of stratification of the egg after centrifuging has a direct bearing on problems concerning the kind of cleavage that may be expected when the top of the egg is separated from the bottom. Both Lillie and Wilson observed that these "fragments" divide at the first cleavage into unequal parts as does the normal egg. Wilson studied the later cleavage as well, finding that it too repeats the normal pattern. I have obtained similar results. Both Tyler (1930) and I report much variability in the cleavage of centrifuged eggs and fragments.

Now, if the fragments represent any part of the original egg the problems are different from those that arise if the dividing fragments represent only the tops of the egg when there is little or no



rearrangement of the materials to be expected. Furthermore, my own results indicate that the tops are from the lightest region of the egg, which is not, in most cases, the true pole that lies to one side of the center of this region. As a result the antipole of the egg, that lies exactly opposite the true pole, both in centrifuged and normal eggs, may sometimes be included in the fragment especially in the large ones. When this happens a "yolk lobe" region may be in part included in such fragments. In fact, I have found many cases when at the first cleavage of large tops a small lobe appears on the larger blastomere. In very small tops, however, no lobe is present, as a rule, as reported also by Wilson. On the other hand if the eggs fell at random, or turned over in the mass of eggs centrifuged in water, and if the polar lobe is a predetermined region of the egg, then one would expect, frequently, to meet with yolk lobes on clear fragments containing the hyaloplasm that came from the antipolar hemisphere of the egg. Cases of this sort have not been reported. In fact we do not know how such antipolar fragments would divide, even if they contained all of the hyaloplasm of the egg.

#### **Is the Excentric Position of the Germinal Vesicle Random or Constitutional?**

If the true pole of the egg be accepted as present before maturation (and there is structural evidence to show that this is the case) the excentric position of the egg nucleus may be supposed either to be a random position in the polar hemisphere, determined perhaps by extrinsic factors (gravity or oxygen supply, for example), or a predetermined one in the egg itself. Either assumption is consistent with the observations. Certain consequences of the position of the nucleus call however for further consideration. Since it has been shown that the first cleavage passes through the center of the area formerly occupied by the egg nucleus (and the true pole), this region becomes the plane that divides the future dorsal from the ventral material of the egg. Hence on the first assumption (extrinsic determination) this axis of the egg is not a predetermined one in the constitution of the egg. On the second assumption the nucleus lies in a predetermined axis. There is at present no way in which one can decide between these alternative interpretations.

There is another consideration that has a bearing on this problem. It has been shown for the eggs of certain animals that the entrance point of the sperm determines the position of the first cleavage plane, and that this point and the egg axis determine the plane that corresponds with one of the major axes of the embryo (right-left or dorso-ventral). Unless there is a predetermined side of the egg, or

a preference side, on which the spermatozoon enters, one of its axes is determined by an extrinsic factor. In some other kinds of eggs, as in the insects for example, the unfertilized egg has a dorso-ventral and right-left form that agrees with that of the future embryo. To what extent its form is determined by the enclosing membrane, or by the follicle cells which surround it during its growth, or by the constitution of the protoplasm itself, is unknown. In some of these cases the spermatozoon enters the egg through a micropyle that lies somewhat on the ventral side of the egg. Whether the enforced entrance of the sperm on a predetermined side plays any role in the axial determination, or merely agrees with it is also unknown.

Now if in *Chaetopterus* the entrance point of the spermatozoon is a factor in determining the axes, it must be assumed to enter on one preferred side that lies in the dorso-ventral axis. On the other hand if it is not a factor then its entrance point is not determinative. There is some evidence that bears on this point. In 1930 Morgan and Tyler studied the relation of the entrance point of the spermatozoon to the location of the first cleavage plane. They did not find a very close agreement between the two. "In 48 eggs there was a fairly strict coincidence; in 35 eggs the entrance point was less than  $45^\circ$  from the plane of the first division. . . . In 33 eggs it was more than  $45^\circ$  and less than  $90^\circ$  degrees to the right or left. Thus in only 41 per cent was there a close agreement between the entrance point and the cleavage plane". These observations may not suffice to settle the point at issue, but as far as they go they are in favor of the view that the spermatozoon is more likely to enter the egg on one side than on the other. This would mean that in this respect also there is a dorso-ventral predetermination in the constitution of the egg.

It has been shown by Lillie (1906) that in eggs that have been partially or completely inverted on the centrifuge that the polar lobe may contain only the oil or the hyaloplasm at the time of the first cleavage. I also have seen occasionally, cases of this sort, and in *Ilyanassa* I have by deliberately inverting the eggs during centrifuging been able to produce a considerable proportion of such lobes. The latter results demonstrate that the formation of the antipolar lobe is not a result of a repetition of the normal pattern of the egg, but is due to a predetermined region of the egg, and since in the case under discussion the interior materials of the lobe may be altered by centrifuging it follows that the formation of the lobe is concerned with the surface materials or surface structure of the egg. This conclusion is in agreement with the results of cutting the egg of *Chaetopterus* in two parts along the true equator. Whitaker and Morgan (1930) and Wilson (1929) have reported that the polar half

does not then form a lobe when it cleaves, while the antipolar half does form a lobe. It is, therefore, more probable that the antipolar lobe is a predetermined region of the egg due to a surface peculiarity of that region, and not to its contents or to its relation to any special configuration of a postulated "ground substance" of the interior of the egg that determines the cleavage pattern at this time.

#### Bibliography

- Clement, Anthony C., 1935. The formation of giant polar bodies in centrifuged eggs of *Ilyanassa*. Biol. Bull., 69: 403.
- Conklin, Edwin C., 1915. Why polar bodies do not develop. Proc. Nat. Acad. Sci., 1: 491.
- 1916. Effects of centrifugal force on the polarity of the eggs of *Crepidula*. Proc. Nat. Acad. Sci., 2: 87.
- 1917. Effects of centrifugal force on the structure and development of the eggs of *Crepidula*. Jour. Exper. Zool., 22: 2.
- Lillie, Frank R., 1906. Observations and experiments concerning the elementary phenomena of embryonic development in *Chaetopterus*. Jour. Exper. Zool., 3: 154.
- 1909. Polarity and bilaterality of the annelid egg. Experiments with centrifugal force. Biol. Bull., 16: 54.
- 1909. Karyokinetic figures of centrifuged eggs; an experimental test of the center of force hypothesis. Biol. Bull., 17: 101.
- Morgan, T. H., 1933. The formation of the antipolar lobe in *Ilyanassa*. Jour. Exper. Zool., 64: 433.
- 1935a. Centrifuging the eggs of *Ilyanassa* in reverse. Biol. Bull., 68: 263.
- 1935b. The separation of the egg of *Ilyanassa* into two parts by centrifuging. Biol. Bull., 68: 280.
- 1935c. The rhythmic changes in form of the isolated antipolar lobe of *Ilyanassa*. Biol. Bull., 68: 296.
- and Albert Tyler, 1935. Effects of centrifuging eggs of *Urechis* before and after fertilization. Jour. Exper. Zool., 70: 301.
- and Albert Tyler, 1930. The point of entrance of the spermatozoön in relation to the orientation of the embryo in eggs with spiral cleavage. Biol. Bull., 58: 59.
- Tyler, A., 1930. Experimental production of double embryos in annelids and mollusks. Jour. Exp. Zool. 57: 347.
- Whitaker, Douglas, and T. H. Morgan, 1930. The cleavage of polar and antipolar halves of the egg of *Chaetopterus*. Biol. Bull., 58: 145.
- Wilson, Edmund B., 1929. The development of egg-fragments in annelids. Arch. f. Entw.-mech., 117: 179.
- 1930. Notes on the development of fragments of the fertilized *Chaetopterus* egg. Biol. Bull., 59: 71.
- Yatsu, N., 1904. Experiments on the development of egg-fragments of *Cerebratulus*. Biol. Bull., 6: 123.
- 1910. Experiments on germinal localization in the eggs of *Cerebratulus*. Jour. Coll. Sci. Tokio, 27.

## Chromosomal Chimeras and Polyploidy in *Solanum gracile* Link<sup>1)</sup>

By  
Fuyuwo Kagawa

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### 1. Introduction

In the genus *Solanum*, polyploid plants have hitherto been obtained by a number of investigators (Winkler 1916, Jørgensen 1928, Lindstrom and Koos 1931, Lindstrom 1932, Lindstrom and Humphrey 1933, Noguchi 1933 and others) as adventitious shoots developed from the callus tissue that formed on the cut surface of stems. In these investigations, the cutting was made either at the point of union after grafting the stems of two different species or at the ordinary stems of a species or species hybrids.

In the following pages, the results of decapitation experiments in the writer's materials, in which chromosomal chimeras were produced are reported, together with some other studies in polyploidy in this species.

### 2. Chromosomal chimeras of diploid and tetraploid

The material plants were grown in 1934 from seeds in pots with soil, and the chromosome number<sup>2)</sup> was verified in iron aceto carmine to be 12 in haploid. The same number of chromosomes was reported

1) The material plant for the present study has been cultivated in our garden for some years, and is certainly of foreign origin. But, as its exact taxonomic name could not be made out here, the herbaria were sent for identification to the Royal Botanic Gardens, Kew, England, who informed us that the plant has the closest affinity to *Solanum gracile* Link, which is of South American origin. In the present study this name is provisionally used, although a more definite taxonomic position, possibly a new one, might be given to it later. The writer wishes to express his cordial thanks to the Royal Botanic Gardens, Kew, for their courtesy.

2) In the present study the chromosomes of PMC were observed exclusively in iron aceto carmine, applied to fresh anthers or those fixed with Carnoy's solution and preserved in 70% alcohol.

by de Vilmorin and Simonet (1928) in their *Solanum gracile* Link material. In the writer's experiments, the stems were decapitated at a point about 10 cm above the ground when the plants were about 20 cm high. From the callus formed on the cut surface, adventitious shoots developed abundantly, and when they formed flower buds the chromosomes of PMC were observed for each shoot.

Thus, about 80 shoots adventitiously developed were examined in the late spring of 1934, and in two of them, which were obtained respectively from Individuals No. 5 and No. 33, 24 was verified as the reduced number.

In Fig. 1, Ind. No. 5 is shown. This plant, when young, was decapitated at point P, and two adventitious buds were formed each



Fig. 1. Photograph of Individual No. 5 with a chimeral shoot. ca. 1/8 natural size. Photographed in late summer 1934. Explanation in text.

of which developed to a vigorous shoot as may be seen in the photograph. The PMC obtained from shoot A, seen at the right in the photograph, showed 24, the tetraploid number of chromosomes in heterotypic and homotypic metaphases, while those from shoot B at the left showed 12, the diploid number in the same stages.

The shape of the leaves and flowers in shoots A and B was similar, and their size differed more or less within each shoot. Generally speaking, however, those in shoot A were larger

and coarser than those in shoot B, and the green colour of the leaves was darker in the former than in the latter. Fig. 2 shows the leaves and flowers of the largest class from each shoot. In several such leaves from both shoots, the size of stomata was measured, and it was found larger in shoot A than in shoot B. The average size of stomatal apparatus (length of guard cells  $\times$  their breadth) as

*the leaf*  
*3/3*  
*A.T.*

calculated from 300 stomata was  $1269.6 \mu^2$  in the former, and  $840.4 \mu^2$  in the latter. The hairs on the leaves and stems were larger in shoot A than in shoot B.

Thus, in shoot A, the sizes of some of the organs and cells are much larger than those in shoot B. Similar conditions have generally been reported by investigators in other examples of autotetraploidy.

The formation of fruits and seeds in shoot A, observed in the fall of 1934, was much less compared with that in shoot B and ordinary diploid plants, although in the shape and size of fruits there was no clear difference.

We shall next turn to the shoot from Ind. No. 33, in which 24 was verified as the reduced number. In this shoot, the leaves

and flowers were rather similar to those of ordinary diploid plants, and the tetraploid characteristics that were found in shoot A of Ind. No. 5 were not markedly observed in these organs. Its fruits and seeds were formed in the fall of 1934 abundantly, contrary to shoot A of Ind. No. 5, its fertility apparently being similar to that in ordinary diploid plants.

In the fall of 1934, several branches of shoot A of Ind. No. 5 and the shoot of Ind. No. 33 in which 24 was found as the reduced number, were cut and propagated asexually in soil in pots. These were overwintered in a green house, and in the spring of 1935 were placed out of doors. Numbers of branches came out from every individual during this period, and their PMC were observed in the summer of 1935.

As may be expected, 24 reduced number of chromosomes was found in the PMC in 5 plants from shoot A of Ind. No. 5. But, quite unexpectedly, 12 was clearly certified in diakinesis, in first and second metaphases as the reduced number in some flower buds obtained from two other plants derived asexually from shoot A of Ind. No. 5, and in one plant from the shoot of Ind. No. 33, in which the tetraploid number was found the preceding year.

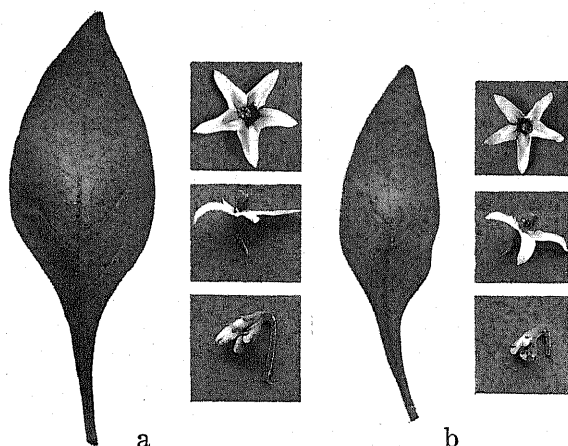
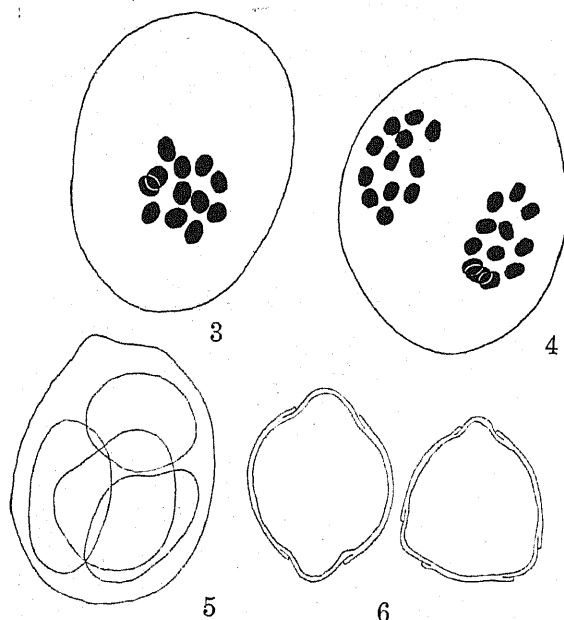


Fig. 2. Leaves and flowers of tetraploid and diploid. a, tetraploid. b, diploid. ca. 4/5 natural size.

In view of these facts, it must be concluded that shoot A of Ind. No. 5 and the shoot of Ind. No. 33, from both of which 24 was verified in 1934 as the reduced number, were really not the tetraploid shoots, but chromosomal chimeras that constituted of both tetraploid and diploid tissues. To the modes of their arrangement we shall come later.

### 3. Pollen formation in diploid and tetraploid

The pollen formation in the anthers obtained in 1934 from shoots A and B of Ind. No. 5 will now be described. In shoot B, 12 was shown as the reduced number in the first as well as in the second metaphase (Figs. 3, 4). Meiosis proceeded regularly, and usually 4 microspores were formed from a PMC (Fig. 5), though rarely 5-6 such were formed owing to 1-2 lagging chromosomes appearing rarely in first and second anaphase and telophase. The proportion



Figs. 3-6. Diploid. 3, first metaphase, 12 bivalents. 4, second metaphase, 12 chromosomes. Polar views.  $\times 1700$ . 5, tetrad.  $\times 1250$ . 6, pollen grains.  $\times 700$ .

of PMC in which apparently the normal tetrad was brought about, was 97.0% (609 in 628). The proportion of good pollen grains was 88.2% (7,149 in 8,105), the remainder being empty or shrunken. Mature pollen grains<sup>1)</sup> of normal structure were usually provided with 3, occasionally 2, germ pores (Fig. 6), while those having 4 were not detected. The average diameter<sup>2)</sup> of pollen grains of normal structure calculated from 358 grains was  $26.2 \mu$ .

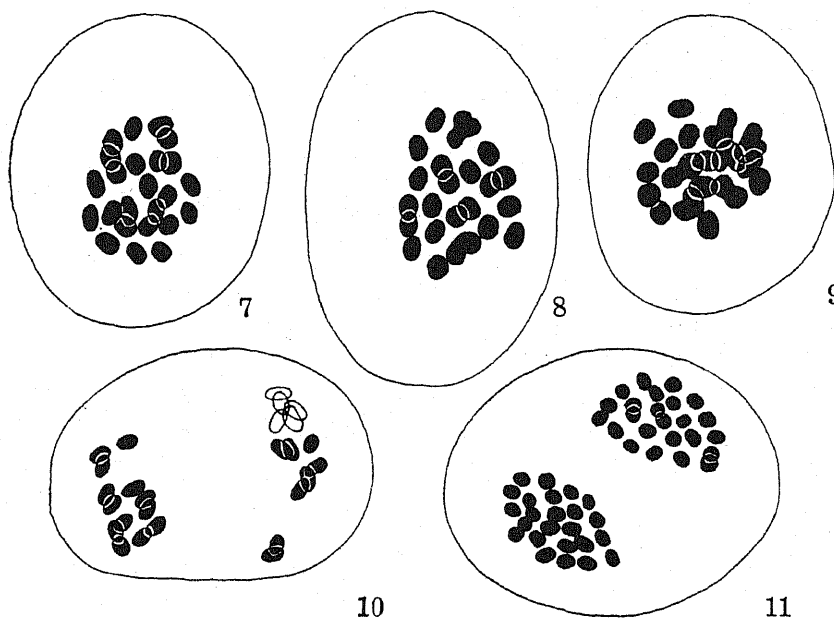
1) The pollen grains were observed in lactic acid.

2) The pollen grains may appear, when seen from a certain angle, to be either spindle shaped or triangular according to the number of germ pores, as the surface of the grain somewhat projects at the places where the germ pores are located. But, if they were observed from an other angle, they may appear almost spherical, if the germ pores are seen above or below the grain surface under the microscope. Such pollen grains showing spherical shape were selected. and their diameter was measured.



In tetraploid PMC from shoot A, 24 bivalents were observed at first metaphase (Fig. 7), but occasionally certain numbers of quadrivalents were observed together with bivalents. In Figs. 8 and 9, 2 and 6 quadrivalents and respectively 20 and 12 bivalents are observed.

At late metaphase, 1-3 univalent chromosomes were often observed away from the equatorial plane. At late anaphase, some univalents were often found located at the periphery of the PMC, separated from the anaphasic groups of chromosomes (Fig. 10). The lagging univalents were almost always found at late anaphase and telophase, and were observed split there.



Figs. 7-11. Tetraploid. 7-9. First metaphase. Polar views. 7, 24 bivalents. 8, 2 quadrivalents and 20 bivalents. 9, 6 quadrivalents and 12 bivalents. 10, first late anaphase: 2 chromosomes are apart from two main groups of chromosomes. Only the chromosomes clearly observable are drawn in main groups. Side view. 11, second metaphase, 24 chromosomes.  $\times 1700$ .

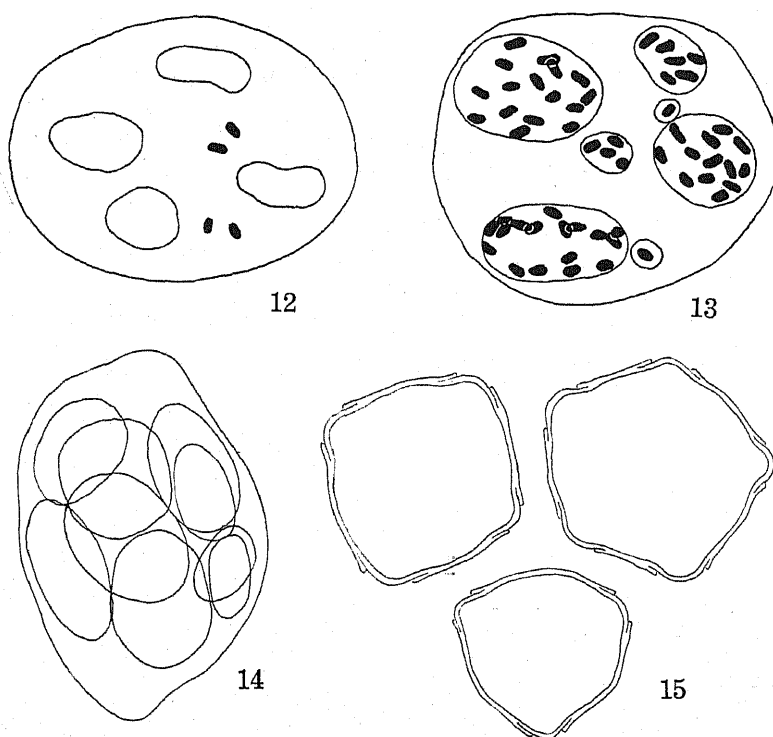
At telophase, the chromosomes, instead of being arranged in two compact groups at the heterotypic poles, were located rather loosely in a comparatively larger spaces in the polar regions. Consequently, some chromosomes located at the periphery of the groups occasionally formed later one or more extra nuclei separated from the two main telophasic nuclei. The extra nuclei were also formed derived from lagging chromosomes.

At second metaphase, 24 chromosomes were found (Fig. 11). But, very frequently a small number of chromosomes appeared



separated from the two metaphasic plates, which was probably brought about by the irregular distribution of chromosomes in the first division. At late anaphase and telophase also, the chromosomes separated from the main groups and the laggards were quite often observed (Fig. 12).

Usually 4 groups of chromosomes were found in a PMC at second telophase. But in most groups, the chromosomes were distributed in comparatively larger spaces and not compactly arranged. And a nuclear membrane did not develop surrounding all the chromosomes of each group, but the chromosomes located at its periphery or somewhat away from it, or those which were lagging behind, occasionally formed one or more extra nuclei (Fig. 13).



Figs. 12-15. Tetraploid. 12, second late anaphase: some chromosomes are lagged behind or located apart from the main groups of chromosomes in cytoplasm. Side view. 13, second telophase showing extra nuclei.  $\times 1700$ . 14, polyspory.  $\times 1250$ . 15, pollen grains.  $\times 700$ .

Owing to the foregoing irregularities in chromosome behaviours in both divisions, polyspory resulted in almost all PMC (Fig. 14). The proportion of PMC in which polyspory was observed was 96.3% (881 in 915), the highest number of microspores formed in a PMC

being 13. The proportion of good pollen grains was only 12.8% (1,281 in 10,005). The mature pollen grains of normal structures were usually provided with 4 germ pores, sometimes 3 and, rarely, 5 (Fig. 15). Those having 2 germ pores were not met with. Their average diameter calculated from 358 grains was  $31.9 \mu$ .

#### 4. Triploid and its pollen formation

In the spring of 1935, the seeds obtained by open pollination from the shoot of Ind. No. 33 in which the tetraploid number of chromosomes was observed in 1934, were sown and the young plants that emerged decapitated. In one individual, only an adventitious shoot had formed showing tetraploid characteristics in the external characters of the leaves and flowers. The tetraploid number 24 was also observed in their PMC. It is not certain, however, whether the shoot of this individual had formed adventitiously on the tetraploid plant without duplication of chromosomes, or whether it had formed on the diploid plant as a tetraploid shoot in which the chromosomes were doubled. Both these possibilities may be conceivable in view of the constitutional nature of the shoot of Ind. No. 33, from which this individual was derived.

This individual was overwintered in a green house, and many branches came out until the summer of 1936. A number of chromosome countings were made on different branches, and the tetraploid number 24 was always detected as far as the observation up to present is concerned. This plant may probably be a true tetraploid.

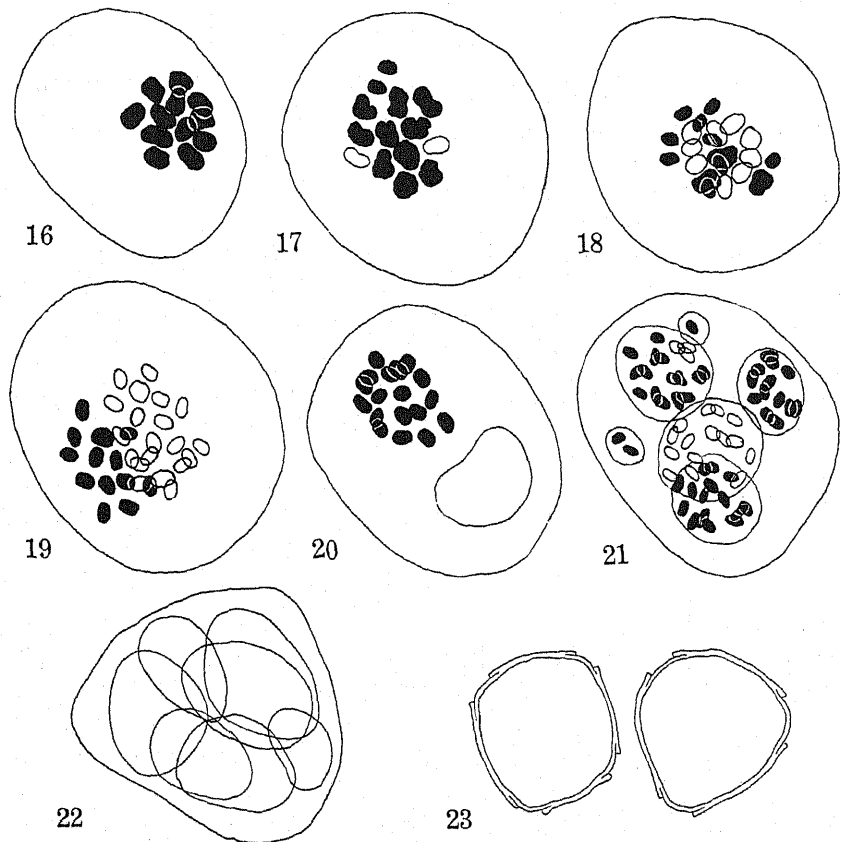
In 1935, this individual was used as the maternal plant and the pollen of diploid plants was pollinated, and four  $F_1$  plants were obtained together with several offsprings in which 24, the tetraploid number was observed.<sup>1)</sup> In  $F_1$  the external characters of the leaves and flowers and the size of stomata were mainly intermediate between diploid and tetraploid.

The chromosome number of  $F_1$  should be 36 in a somatic count. In the first meiotic metaphase of the PMC, 12 trivalents were sometimes clearly observed (Fig. 16), but more frequently, different numbers of bivalent and univalent chromosomes were observed together with trivalents. These three kinds of chromosomes were distinguishable in many cases from each other by their size. In Fig. 17, 10 trivalents, 2 bivalents and 2 univalents are observable, while in Fig. 18, 3 trivalents, 9 bivalents and 9 univalents are shown.

1) As to the cause of the formation of tetraploid progeny, which was not the self fertilization in the mother plant, we hope to deal with it in a following opportunity.

At the first anaphase, the components of trivalents passed on to poles as univalents, but some bivalents which were made probably of the two component univalents of trivalents were also observed on their way to the poles. In Fig. 19, which is a polar view of this stage, 1 bivalent plus 12 univalents and 2 bivalents plus 18 univalents are observed on different optical planes passing to poles. The numbers of chromosome elements going to a pole that were counted in polar views of first anaphase were 12-19. At first telophase 1-4 univalent chromosomes were frequently found lagging behind and split there.

At the second metaphase, 12-18 chromosomes were observed (Fig. 20), and occasionally some of the chromosomes were located separated from the main metaphasic groups. At the second late telophase, we frequently found extra nuclei derived from some chromosomes that were lagging behind or were located away from the main



Figs. 16-23. Triploid. 16-18, first metaphase. Polar views. 16, 12 trivalents. 17, 10 trivalents, 2 bivalents and 2 univalents. 18, 3 trivalents, 9 bivalents and 9 univalents. 19, first anaphase. Polar view. 20, second metaphase with 18 chromosomes. Polar view. 21, second telophase, extra nuclei.  $\times 1700$ . 22, polyspory.  $\times 1250$ . 23, pollen grains.  $\times 700$ .

telophasic groups of chromosomes (Fig. 21). So that polyspory resulted in the majority of PMC (Fig. 22), the highest number of microspores formed in a PMC being 11. The proportion of PMC showing polyspory was 92.4% (501 in 542). Dyad cells were never met with at tetrad stage.

The majority of pollen grains were empty and shrunken, and the proportion of good pollen grains was 13.0% (1,447 in 11,166). The normally developed pollen grains were provided with 3-4 germ pores (Fig. 23), a number intermediate between those observed in diploid and tetraploid. Pollen grains showing 2 or 5 germ pores were not detected. The average size of normally developed pollen grains calculated from 358 grains was  $27.7 \mu$  in diameter, a size intermediate between those of diploid and tetraploid.

### 5. Discussion

In the decapitation experiments hitherto reported by investigators, either the diploid or the tetraploid number of chromosomes was, or seems to have been, exclusively found in an adventitious shoot developed from the callus tissue on the cut surface. Some, however, have reported the examples in which the organs of diploid aspects appeared in a tetraploid shoot formed in this way, although a chromosome counting in such regions that showed a diploid nature was not made.

Winkler (1916) obtained tetraploid individuals of *Solanum nigrum*, that were asexually derived from adventitious shoots developed from the callus tissue on a cut surface, made at the point of union after grafting the stems of *S. Lycopersicum* and *S. nigrum*. He observed in such tetraploid *S. nigrum* that, in certain leaves there appeared the shape, colour and anatomical structures that were characteristic of diploid *S. nigrum*, from which he inferred that the diploid cells were contained in such leaves, although the chromosome number in them was not observed. He further considered that the reduction in chromosome number might have occurred in the original somatic cells, and that by the diploid cells derived from these cells such diploid regions were formed. He considered it possible that a part of a flower, an anther for example, may become diploid and produce pollen grains containing the haploid number of chromosomes.

According to Jørgensen (1928), a triploid tomato shoot was formed as a somatic sport on a plant that was regarded from its morphological aspects to be diploid, though on its formative cause no conclusion was made. In this connection, a possibility was stated that, trinucleate somatic cells may be formed in a diploid plant resulting in hexaploid somatic cells, and that the triploid cells may be

formed through reduction of chromosome number occurring in such cells, from which a triploid shoot may be derived.

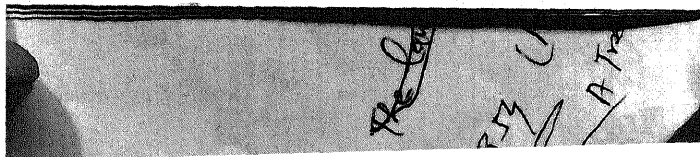
Jørgensen (1928) obtained in *S. nigrum* by the method of decapitation a periclinal chimera of diploid and tetraploid tissues, together with tetraploid plants. It was reported that in this chimera, the diploid core was covered by at least two layers of tetraploid skins, although the precise descriptions of this chimera, especially of its chromosomes, were not given. He also pointed out the possibility of the formation of chromosomal chimera in tomatoes. Lindstrom and Koos (1931) also obtained through the decapitation method applied to diploid plants, some tomato plants that were apparently sectorial chimeras of diploid and tetraploid tissues, although the chromosomes were not examined.

It seems quite natural that the growing point of an adventitious shoot developing from the callus may consist of diploid and tetraploid tissues, located side by side sectorially, or arranged periclinally, or mixed together in a complex manner. The cases of adventitious shoots formed by tetraploid cells alone might be special occurrences.<sup>1)</sup>

A discussion of reduction of chromosome number in somatic cells, which may play a certain rôle in the formation of chromosomal chimeras, is outside the purpose of the present paper. However, the appearance of diploid tissues or organs in the so-called tetraploid shoots might naturally be expected if such shoots were not really the tetraploid, but of the chimeral nature developed adventitiously from diploid and tetraploid cells in the callus.

In the late spring of 1934 when the plants were young and small, shoot A of Ind. No. 5 showed in their leaves and flowers morphological characteristics of tetraploid plants, while in the shoot of Ind. No. 33 in which 24 was found as the reduced number, the leaves and flowers were rather similar to those of the diploid plants, in marked contrast to shoot A of Ind. No. 5. This seemed odd at that time. In this connection, it might be supposed that, the leaves and flowers of shoot

1) In our laboratory, the analogous chimera shoots were obtained in this plant *S. gracile* Link by S. Sakamoto and Y. Araki during the years 1935-1936 (unpublished). In the spring 1935, several adventitious shoots of tetraploid outlook were obtained, by the decapitation callus method, on diploid stocks, and 24 the tetraploid number of chromosomes was observed in PMC from each of these shoots. These plants were overwintered in a greenhouse without being propagated by cutting, and in the summer of 1936, the chromosomes were again observed in PMC from the same shoots. In two shoots on different stocks, the tetraploid number was exclusively found in a number of countings, while in one other plant 12 the diploid number was counted in 9 flower buds obtained from different positions on the shoot, in which the tetraploid number was detected the preceding year. This shoot must evidently be a chromosomal chimera.



A of Ind. No. 5 were formed at that period mostly of the tetraploid tissue, while the diploid tissue was not yet properly developed, though such was contained in some parts of the shoot. On the other hand, the shoot of Ind. No. 33 from which the tetraploid number was ascertained might have possibly been constituted at that time as a periclinal chimera, the subepidermal layer of which was made of the tetraploid cells. In periclinal chimeras the external characters may be more or less intermediate between tetraploid and diploid.

In the case of an adventitious shoot that started from the callus tissue on the decapitated surface, it is possible that the diploid and the tetraploid tissues are arranged in various ways, sectorially, periclinally, or in some other complex manner. It is also probable that the modes of arrangement of different kinds of tissues change in some branches or branchlets during cultivation for long periods. Jørgensen and Crane (1927) obtained a number of *Solanum* chimeras as the adventitious shoots started from the callus tissue on a cut surface, that was made at the point of union after grafting the stems of two different species. According to these authors, the periclinal chimeras which were obtained in this way, and which were constituted of two kinds of tissues from different species, produced pure shoots constituted exclusively of either one or the other kind of the component tissues, when propagated asexually and during cultivation for two years.

The fertility in the shoot of Ind. No. 33 which showed the tetraploid number at late spring in 1934, was much higher than that in shoot A of Ind. No. 5, at the fall of the same year. It is possible that in this individual, the diploid branches or the chimera branches having diploid cells in the subepidermal layer, grew abundantly after the chromosomes were examined, resulting in greater fertility, which was owing to the regular meiosis in diploid PMC and the subsequent formation of good pollen grains.

In the branches or branchlets, from which the PMC showing the diploid number was detected in 1935, the subepidermal layer must naturally have been composed of diploid cells, although the branches or branchlets themselves might have been constituted exclusively of diploid cells or of diploid and tetraploid cells in chimera arrangement of different modes.

In order to ascertain how the branches or branchlets of different chromosome contents were distributed in a plant in 1935, it might be useful to compare the external characters of the leaves and flowers. But, unfortunately this was not possible, as the plants grew poorly and were stunted, which was largely the result of lack of sufficient temperature and illumination in the green house, malnutrition in

small pots, and frequent spraying of insecticides. Ind. No. 5 itself was also overwintered under the same conditions, and later placed outdoors in 1935. And its shoots A and B did not show, owing mainly to the same reasons, any distinct difference in the characters of leaves and flowers in the green house and outdoors in 1935, although it was clearly observed the preceding year.

In view of the foregoing results, we can not always regard the adventitious shoots with large flowers and large and dark green leaves as true tetraploid plants, even should the meiosis in some anthers obtained from such shoots show the tetraploid number of chromosomes. On the other hand, if a plant grown from seed which had formed on such adventitious shoot shows the tetraploid number of chromosomes, it may then be concluded that it is a real tetraploid plant. This must be noticed in the course of genetical and breeding works which may be undertaken in connection with the decapitation callus method.

## 6. Summary

1) In *Solanum gracile* Link, the chromosomal chimeras composed of diploid ( $n=12$ ) and tetraploid ( $n=24$ ) tissues were obtained by the decapitation callus method applied to diploid plants, and the occurrence of such chimeras and the modes of arrangement of the two kinds of tissues were discussed.

2) Triploid plants were obtained by crossing an individual, which was probably a true tetraploid, with the pollen of diploid plants. In tetraploid and triploid, the course of pollen formation resulting in a high degree of pollen abortion was studied.

Sept. 1936, Utsunomiya Agricultural College

## 7. Literature cited

- Jørgensen, C. A. 1928. The experimental formation of heteroploid plants in the genus *Solanum*. Jour. Gen. Vol. 19.  
— and Crane, M. B. 1927. Formation and morphology of *Solanum* chimeras. Jour. Gen. Vol. 18.  
Lindstrom, E. 1932. A fertile tetraploid tomato. Jour. Hered. Vol. 23.  
Lindstrom, E. W. and Koos, K. 1931. Cyto-genetic investigations of a haploid tomato and its diploid and tetraploid progeny. Amer. Jour. Bot. Vol. 18.  
— and Humphrey, L. M. 1933. Comparative cyto-genetic studies of tetraploid tomatoes from different origins. Genetics, Vol. 18.  
Noguchi, Y. 1933. Formation of tetraploid tomatoes by the decapitation-callus method. (Japanese). Bot. and Zoolog. (Tokyo) Vol. 1.  
Vilmorin, R. de et Simonet, M. 1928. Recherches sur le nombre des chromosomes chez les Solanées. Verhandl. V. Intern. Kongr. f. Vererb.-wiss.  
Winkler, H. 1916. Über die experimentelle Erzeugung von Pflanzen mit abweichenden Chromosomenzahlen. Zeitschr. f. Bot. Bd. 8.

**Correspondences Between Linkage Maps and Salivary Chromosome  
Structure, as Illustrated in the Tip of Chromosome 2R  
of *Drosophila melanogaster***

By

**Calvin B. Bridges**

Carnegie Institution of Washington, and the Wm. G. Kerckhoff  
Biological Laboratories, California Institute of Technology,  
Pasadena, California

**Introduction**

During the past two years the emphasis in the *Drosophila* work on the structure and workings of the germ plasm has shifted from analysis by breeding methods to analysis by microscopical observations. Before the importance of the salivary chromosomes was pointed out by Painter (Painter 1933), more could be learned about the distribution and behavior of chromosomes and about their internal differentiation by "counting flies" than by looking at the germ cells themselves. Genetical analysis, through studies of mutation, linkage relations and of aberrations such as non-disjunction, inversion, deficiency and translocation, forged far ahead of the checking that was done through examination of the tiny metaphase chromosomes. But it must not be forgotten that it was this sound foundation, with the establishment of the serial order of the genes within the several chromosomes and with an approximation to their real positions in those chromosomes, that gave instant meaning to the seriation of bands along the salivary chromosomes.

The standard maps of the normal salivary chromosomes (Painter 1934, 1935; Bridges 1935) have been indispensable in analyzing the interrelations between the chromosome sub-structures and the linkage maps. But, although correct so far as they go and satisfactory for general orientation, they are inadequate for the precision needed for close point-to-point homologies. A revision of the salivary maps is now under way which will show approximately half again as many bands and will represent more correctly their relative intensities, individual characteristics and their nearly universal appearance in pairs as "doublets".

The correspondences between the cross-banding, the structural features and the distances along salivary chromosomes with the loci, regions and distances along the linkage maps have been studied in detail by use of the right end of chromosome 2R of *Drosophila melano-*



*gaster*. Homologous regions and points in the two series of loci and banding have been established and local differences and peculiarities in the frequencies both of crossing-over and of mutation have been demonstrated. The interconnections have been found through study of the breakage points of five inversions, two translocations and four deficiencies all having one or both breaks within the short section from near the locus of "humpy" to the tip of 2R. In this same region several other aberrations are known and when they also have been analyzed the correspondences should be still finer in grain.

### The loci of mutations and their spacing on the linkage map

Linkage maps are constructed from genetical data on the frequencies of recombination when two or more pairs of alternative characters are involved in the same cross—preferably the "back-cross" or "test-cross". The test-cross is the type of mating in which a multiple heterozygote is crossed to the corresponding multiple recessive, and is so called because the progeny are in classes which are a direct replica (aside from differential mortality) of the gametic series of the heterozygous parent. For example, if the recessive brown eye is crossed to the recessive speck and the wild-type doubly heterozygous female ( $bw +/+ sp$ ) is tested by the double recessive  $bw sp$  male the test-cross progeny are found (averages of many trials) to be:  $bw = 48.75\%$ ,  $sp = 48.75\%$ ,  $bw sp = 1.25\%$  and wild-type =  $1.25\%$ . The new combinations total  $2.5\%$ . Recombination of linked characters is brought about by crossing-over of the chromosome threads at some point between the loci of the two mutants. Hence for each recombination one crossover must have occurred. But if the loci are far apart two separate crossovers may occur between them and the second would restore the original combination. Thus, for short intervals percentages of recombination are directly equal to cases of crossing over per average hundred gametes tested, but for longer intervals a table of experimentally determined multiple crossing-over frequencies is needed to convert recombination percentages into crossing-over units or linkage-map distances.

In the upper line of Figure 1 is given the linkage map of the extreme right end of chromosome 2R. This map is revised according to the latest information derived from much unpublished work. These mutants are briefly described below, in alphabetical order of the symbols of the loci, giving symbol, locus name, finder, date of discovery, chromosome and locus reference to publications and figures, facts concerning origin, description of phenotype and associated characteristics. Finally is given a valuation or "Rank" designation,

RK 1 denoting the most useful loci, RK 2 those nearly as good, RK 3 good mutants not accurately mapped, RK 4 poor mutant phenotypes and RK 5 poor phenotypes not accurately mapped. The mutants Minute-1<sup>2</sup> and Minute-33a were found and located by Dr. J. Schultz, who also determined that the locus of minus is to the right of brown.

- a—arc. C. B. Bridges, May 24, '12. 2-99.2. Pub. Bridges and Morgan 1919; fig. 4, plate 7. Wings broader, bent downward in slight even arc and edges drawn down to diamond shape. Crossveins closer together (ratio crossvein length : inter-cv dist. = 1 : 1.9). Classification perfect after practice. RK 2.
- abb—abbreviated. C. B. Bridges, Apr. 6, '28. 2-105.5. Unpublished. Macrochaetae smaller, especially posterior scutellars. Hatches slightly later than normal. Viability only slightly reduced. Classification hard and uncertain in early hatches, improves with age of culture. Three intensifiers found, of which "shrunk" (2-2.3) confers perfect classification. RK 4; with shr, RK 2.
- ba—balloon. T. H. Morgan, Nov. '10. 2-107.4. Pub. Bridges and Morgan 1919; fig. 1, plate 7. Wings at first inflated with blood, leaving blisters and vesicles. Venation weak; straggly, plexus-like. Wings smaller, warped, discolored, divergent. Sensitive to temperature: above 25° RK 4; 19° or below, classification easy, RK 2; ba/Px, below 19°, RK 2.
- bs—blistered. C. B. Bridges, Nov. 16 '11. 2-107.3. Pub. Bridges and Morgan 1919; text-fig. 74. Wings blistered, smaller, pointed. Venation thicker, plexus-like, especially branches from and parallel to L5 beyond CV2, semidominant "free-vein effect" here. Eye-color softened. Sensitive to temp.: at 25° RK 4; at 19° and below, RK 2. (More extreme allele = bs<sup>2</sup>. C. B. Bridges, Nov. 24, '25. RK 2; bs<sup>2</sup>/Px below 19°, RK 1.)
- bw—brown. G. H. M. Waaler, Oct. 15, '19. 2-105.0. Pub. 1921. Eye-color light brownish pink, brilliant and translucent. Testes colorless. Malpighian tubules nearly colorless (∴ classifiable in larvae). Reoccurs with quite high frequency; also numerous alleles. RK 1.
- hy—humpy. C. B. Bridges, Oct. 22, '18. 2-93.3. Unpublished. Thorax strongly ridged, with "commas" at front and 2 pairs deep vortices. Wings obliquely truncated to half length. Highly sterile in both sexes. Viability low and erratic. Classification easy (but px no good in hy). RK 2 (location strategic).
- ll—lanceolate. C. B. Bridges, Apr. 3, '23. 2-106.7± (order of ll and mr not determined). Unpublished. Wings narrowed at the tip and slightly divergent. RK 4. (More extreme allele = ll<sup>2</sup>, C. B. Bridges, Apr. 25, '23; RK 2).
- lNS—lethal-Nova Scotia. C. B. Bridges, Oct. 31, '25. 2-107.0—to right of l2ax, to left of sp). Unpublished. Lethal in larval stage at 2 mm length, by weak and abnormal development of tracheae and other chitinized parts. Present only in Inversion-Nova Scotia px sp stock. RK 4.
- l2ax—(lx)—lethal-2ax. C. B. Bridges, Feb. 28, '19. 2-106.9. Unpublished. Lethal in very early larva stage. RK 4.
- M33a—(M33)—Minute-33a. J. Schultz, Jan. 7, '33. 2-108.0. Unpublished. From X-rayed ♂. Minute-bristles of "slight" type, with excellent viability, fertility and classifiability. Hom. = lethal. (Originally called M115). RK 1. Salivary analysis by Bridges shows deficiency of six

- lines, starting to right of heavy doublet 60 E 1 and ending to left of diffuse doublet which is the last line of subsection 60 E.
- Mc—Minute-c. A. H. Sturtevant, Jan. 7, '20. 2-107.0±. Unpublished. A rather extreme "minute-bristle" type. Lost.
- MI<sup>2</sup>—Minute-l<sup>2</sup>. J. Schultz, Jan. 7, '26. 2-101.2. Unpublished. Minute-bristles of slight type, with excellent viability, fertility and classifiability. Hom. = lethal. Not deficient for any known gene. RK 2. (In some relations RK 1) Salivary banding not detected abnormal.
- mi—minus. R. L. Biddle, Dec. '28. 2-104.7. Unpublished. Bristles nearly as small as hairs. Hairs smaller and fewer. Body size very small. Emergence delayed. Viability low and erratic. Females entirely sterile, males fertile. RK 2-RK 4.
- mr—morula. C. B. Bridges, Mar. 8, '13. 2-106.7 (ll-mr order undetermined). Pub. Bridges and Morgan 1919; plate 10, figs. 3a and 3b. Eye rough. Bristles irregularly reduced in size and number. Abdominal sclerites smaller. Females entirely sterile, with undeveloped ovaries. RK 2. (mr<sup>2</sup>, less extreme allele, C. B. Bridges, Nov. 24, '25).
- pd—purpleoid. C. B. Bridges, Aug. 31, '16. 2-106.4. Unpublished. Eye-color dark pink or maroon; like "purple" but less extreme. Semi-dominant, giving softening of eye color. Separation of hom. from het. by lighter orange "fleck". RK 2.
- pkh—pinkish. C. B. Bridges, July 27, '14. 2-106±5. Pub. Jour. Exp. Zool. 28:337-384. Specific dilutor of w-eosin. RK 4. Lost.
- px—plexus. C. B. Bridges, Aug. 20, '14. 2-100.5. Pub. Bridges and Morgan 1919: text-fig. 80. Venation network of extra veins, especially toward tip and margin of wing. One of the most useful of the second-chromosome characters. RK 1.
- sp—speck. T. H. Morgan, March '10. 2-107.0. Pub. Bridges and Morgan 1919; text-figs. 73a, 73b, 75b, plate 5, figs. 1, 2. Intense black speck in axil of wing. Body color darker, "olive". Best character in right end of 2. RK 1. (More intense allele = sp<sup>2</sup>; C. B. Bridges, June '25; RK 1).

### The banding of the salivary chromosomes

That the salivary chromosomes are differentiated along their lengths by a succession of crossbands is well known. In aceto-carmine preparations the chromosome presents the picture of a faintly staining cylinder with strongly staining crossbands. The crossbands differ from each other markedly in intensity of stain, in the width of the bands, and also in character as a solid cross line or as a line broken into segments or dots. Most lines are sharply defined and hard-edged, while some are diffuse and hazy of outline. In any given place in each chromosome the set of bands is characteristic and constant. However, different treatments in making the preparations (such as variation in the amount of iron, and differences in the length of staining and quality of the carmine itself) cause the relative intensities to appear somewhat different from preparation to preparation.

That the chromosome is differentiated into two distinct substances is plain, not only from the staining or non-staining of specific points but from differences in refractive index of these same parts, whereby in fresh cells the same succession of high-refractive cross bands and low-refractive matrix, or substance between bands, can be seen. Also, under mechanical stress the dark-staining, highly refractive bands reveal themselves as solid, inelastic and disc-like, while the material between bands is very elastic—drawing out to at least three times its lax length without apparent disruptive change in its structure. Finally, the Fuelgen reaction shows that the solid, inelastic, highly refractive, darkly staining cross bands are highly charged with nucleic acid while the inter-band regions are relatively free of nucleic acid.

Intensive microscopical study of the banding has given us maps of the characteristic banding throughout the chromosomes (Painter 1934, 1935; Bridges 1935). In the bottom of Fig. 1 is given a map for the extreme right end of chromosome 2R—corresponding to the part whose linkage map has already been described. This map is revised from that given in Bridges 1935 by a more complete presentation of the faint bands and by a more accurate representation of the specific characteristics of all bands.

The division of many crossbands into dots or segments is an expression of the multiple structure of the giant chromosome as a bundle of chromonemal threads derived through successive divisions of the original threads contributed by the gametes. Usually 16 of these granules can be seen in the bands of *Drosophila melanogaster* "diploid" chromosomes. Where fewer can be seen it is believed the threads are nevertheless divided but are still adherent by virtue of the synaptic action of the homologous parts of the threads. Because of this property of continued synaptic adhesion it may very well be that the visual number of threads is much lower than the actual number, very possibly 256 per haploid strand, as suggested by Hertwig (G. Hertwig 1935).

It would seem that there is a special class of bands (illustrated by 60E1) that is characterized by greater intensity than the majority of bands. Generally these "heavy" bands are clearly doublets, of which both lines have the same high intensity (illustrated by 60D1). They also often form "capsules" by union at their edges, with a clear space between (58A, 59E). But even the medium and faint lines seem to be clearly doublets in the great majority of cases (60A). What the significance of this predominant feature of the banding may be, is problematical. It could be a primary characteristic of band (and gene) structure or it could be a secondary doubled condition, brought

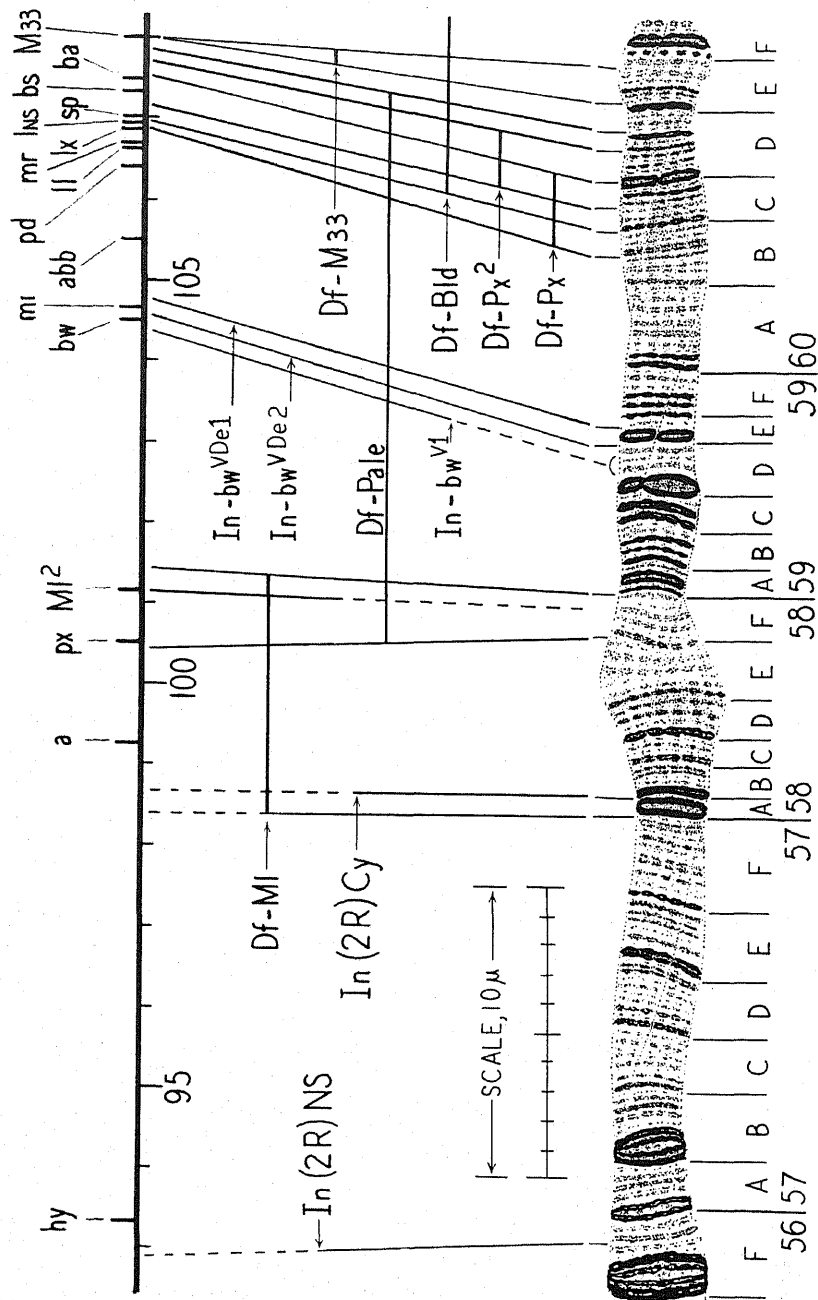


Fig. 1. Homologous points in salivary chromosome and linkage map of tip of 2R.

about, for example, by unequal crossing-over. The probabilities seem fair that the very heavy type of band is to be interpreted as secondary doubling.

As pointed out before (Bridges 1935) there are sections of the chromosomes which in appearance are duplicates of other sections. In 56F and 57B are short groups of heavy bands which seem to be symmetrical reversed "repeats," that is, to consist of the same bands extending in both directions from a central point of symmetry. Furthermore it would seem that these two heavy groups are themselves repeats of each other—that the center of symmetry of this longer reversed repeat is in 57A. Another region suggestive of reversed repetition is in 58E–F.

Sections 58E and F show another characteristic peculiarity—namely, they often are converted into a much swollen light-staining "puff" in which the banding is very hard to see. The light region in the rounded tip of the chromosome (in 60E) also tends in this direction. Thus, sections of the chromosome show variations in the matrix or back-ground structure, as well as in the banding itself.

### **The chromosomal aberrations and their use in homologizing the linkage with the salivary maps**

The most useful category of aberrations for homologizing the two kinds of chromosome maps is deficiencies—the loss of a section of genes. The location of the deficiency on the linkage map can be found by the ordinary linkage tests, since the heterozygous deficiency usually gives somatic alterations constituting a dominant phenotype. Thus, the locus of the deficiency giving rise to "plexate" venation can be located as at a point some 2.4 units to the right of the locus brown and near the extreme tip of 2R. It has been found that deficiencies give relatively very little alteration in the crossing-over frequencies and hence the linkage tests are in themselves very useful when properly interpreted. But for deficiencies there is another and very exact method of location, namely, the pseudo-dominance test for included loci. To illustrate this: the cross of Plexate by brown gives equal numbers of Plexate and of wild-type offspring, showing no pseudo-dominance of brown in the Plexate flies, but the cross of Plexate to speck yields wild-type offspring and Plexate offspring which also show speck. Since speck is a strict recessive this pseudo-dominance means that the deficiency has removed the wild-type allele of speck and that in Plexate flies the character speck is manifest for the same reason that the character yellow shows in the male with its single X-chromosome. With Plexate not only speck but also blistered and balloon and a lethal (INS) prove to be included in the deficiency. Thus, a section of the map from 106.9 to 107.5 and including 4 loci is known to correspond to the deficiency. Examination of the salivary gland chromosomes of the Plexate heterozygote

shows that a section of about a dozen cross lines (counting doublets as 2) is absent. This section extends from line 60B7 (see lower line of Fig. 1) to the right of line 60D1. The break at the right end of the deficiency passes between the two equal lines of the heavy doublet. This fact shows that the parts of heavy doublets can have independent existence and hence can be each a gene.

The other deficiencies studied similarly were Plexate<sup>2</sup>, Minute-33a and two deficiencies derived from translocations, viz. the deficiency from T(2-3)-Pale and from T(1-2)-Blond. The limits of these deficiencies on the genetic and on the salivary map are indicated in Fig. 1. The dominant Minute-1<sup>2</sup>, which had been suspected of being a deficiency, has not thus far been found to have any bands missing.

The inversions do not offer very favorable material for homologizing the two maps, because crossing-over is so reduced in the presence of a heterozygous inversion that it is very difficult to determine where the break comes in the linkage map. But the establishment of the breakage point in the salivary chromosome is easy. Two of the first-found inversions have break-points in this region of 2R, namely In(2R)-Nova Scotia and In(2R)-Curly. The salivary studies by Bridges and Li (unpublished) have shown these points to be as indicated in Fig. 1.

Schultz has studied the break-points of inversions associated with three of the variegated-brown dominants, namely, bw<sup>V1</sup> ("Plum" of Muller) and two brown-variegates found by Demerec in the progeny of X-rayed flies. The locations of these breaks in the salivary map are given by Schultz in the lower line of Fig. 1. The breaks of these three inversions (In-bw<sup>V1</sup> has not as yet been closely determined) come in a narrow region in 59 D-E. The brown-variegates from Demerec enable the locus of minus to be placed to the right of brown and in the heavy capsular doublet in 59E.

For the loci lNS, sp, bs and ba, the limits within which the given gene must lie are determined with fairly high accuracy and narrow range.

### Coefficients of crossing-over

Comparison of the relative positions of given break-points in the two series shows clearly that the seriation is the same in both. However, the relative spacings vary somewhat according to the particular region. The studies of Painter and Bridges showed that the over-all lengths of the different salivary strands (X, 2L, 2R, 3L, 3R and 4) are closely proportional to the lengths of the corresponding linkage maps. We may define as the "normal" ratio of crossing-over this ratio of lengths of linkage maps to lengths of salivary chromosomes. The maps total 66, 108, 105 and 0.2 units, or approximately 279 units.



The salivary strands total  $1180 \mu$  (Bridges 1935). Hence the "normal" ratio is  $1180/279 = 4.2 \mu$  per unit of map distance. Any section of chromosome can be said to have the normal coefficient of crossing-over (one) if it has  $4.2 \mu$  of salivary map (as measured off on the maps of Bridges 1935) to one unit of crossing-over.

Regional differences in coefficient of crossing-over are strikingly apparent when 2R is examined, and are seen in the tip region. Thus, the map distance from px to M33a is 7.5 units, corresponding to  $18.5 \mu$ . The ratio of crossing-over is thus  $2.5 \mu$  per unit of crossing-over, and the coefficient of crossing-over ( $4.2/2.4$ ) is 1.7. That is, in this tip region, crossing-over is approximately 70% freer than in the set of chromosomes as a whole.

There are also local differences within this tip region. Thus, the px-bw map distance is 4.0 units, corresponding to  $7 \mu$ , giving a ratio of 1 unit  $1.75 \mu$  and a very high coefficient of 2.4.

### Coefficients of mutation

A striking feature of the linkage maps is the bunching of mutant loci in certain regions, coupled with the occurrence of long stretches elsewhere relatively free from mutant loci. In the past such local differences have been explained by appeal to local differences in ease of crossing-over—for example, by the assumption of a low coefficient of crossing-over for the regions near the middle of chromosomes 2 and 3. The salivary technique offers a better check on this than did the metaphase groups and it is seen that part of this non-random grouping is indeed due to differences in coefficient of crossing-over. However, the dense group of mutant loci at the end of 2R would be explained by an exceptionally low coefficient of crossing-over for that region, while we have just seen that the contrary is true—that for this tip region from px to M33a crossing-over is about 70% above standard.

Another variable must be appealed to, namely the *coefficient of mutation*. It was only through such salivary studies as the present one that accurate enough information on the localization of mutants in the actual chromosome has been obtained to make it certain that regional differences in mutability exist. In Fig. 1, it can be seen that from the locus of humpy to plexus, is 7.2 units of map distance, corresponding to about  $19 \mu$  of chromosome. Similarly, from px to M33a is 7.5 units of map distance corresponding to  $18.5 \mu$ . That is, the coefficients of crossing-over in these two sections are substantially equal (1.6 and 1.7). But, in the first section are only three mutant loci, while in the second are 15. Since the included lengths of chromosome (together with numbers of crossbands) in these two sec-



tions are substantially equal, we must attribute this striking difference to a mutability of genes approximately five times as high in the second section as in the first. A standard coefficient of mutability may be taken as the total of mutant loci divided into the total length of chromosomes. According to the latest revision of maps by Bridges (DIS-3:21-26) the number of loci of working mutants (probably proportional to the totals of all mutants) is about 280.

Hence the standard incidence of detected workable mutations is at present 1 mutant locus per  $4.2 \mu$  of salivary length. The ratio of mutants to salivary length in the hy-px section is 3:19 or 1:6.3, and in the px-M33a section is 15:18.5 or 1:1.2. That is, in the hy-px section mutability is about  $0.67 \times$  standard, while in the px-M33a section it is  $3.5 \times$  standard.

It should be noted that the coefficient of mutation varies independently from the coefficient of crossing-over. This is readily seen in the two sections just compared, having the same somewhat high coefficient of crossing-over but very different coefficients of mutation—one somewhat lower than normal and the other much higher. Furthermore, the data on which the two measures are based are different in nature—one depending on frequencies of crossing-over between given points and the other on merely the numbers of mutant loci between points.

#### **The interpretation of a low coefficient of detected mutation as a high frequency of duplications or "repeats"**

That the genes of a particular large section of chromosome should differ intrinsically in respect to mutability from those of the general group seems less likely than that secondary effects of some kind are present making regional differences. A regional factor which may very well account for most of the differences detected in the tip of 2R is the prevalence of duplications ("repeats") present in the normal chromosomes. The basal half of 2L is very rich indeed with such duplications, and is also low in coefficient of mutation, considering that the locus of black is (according to unpublished data of Painter) at a point roughly one-third of the distance out toward the tip of 2L. If the genes of a given short section are present in another place in the group (either immediately adjacent or elsewhere in the same chromosome or in a different chromosome) then this duplication serves as a buffer against the manifestation of recessive mutants with loci in the given section. A recessive has to occur not only in that section but also in the duplication before it can be manifest. Hence any region rich in sections duplicated elsewhere will have a low coefficient of mutation. Even the incidence of dominants should be

lowered, since in normal diploids many dominants give somatic effects only slightly above the threshold of detection and since the class of dominants from deficiencies would be reduced or absent. Observationally there seems to be a "reversed repeat", extending from the beginning of 56F into the middle of 57B, and another in the puff of 58E.

#### Literature cited

- Bridges, C. B., 1935. Salivary chromosome maps, with a key to the banding of the chromosomes of *Drosophila melanogaster*. Jour. Hered. 26: 60-64.
- 1935. Linkage maps. *Drosophila* Information Service 3: 21-26.
- Hertwig, G., 1935. Die Vielwertigkeit der Speicheldrüsenkerne und Chromosomen bei *Drosophila melanogaster*. Z.i.A.V., 70: 496-501.
- Painter, T. S., 1933. A new method for the study of chromosome rearrangements and the plotting of chromosome maps. Science 78: 585-586.
- 1934. Salivary chromosomes and the attack on the gene. Jour. Hered. 25: 464-476.
- 1935. The morphology of the third chromosome in the salivary gland of *Drosophila melanogaster* and a new cytological map of this element. Genetics 20: 301-326.

## Microsporogenesis in Diploid and Triploid Types of *Lilium tigrinum* with Special References to Abortions

By

Clyde Chandler, W. M. Porterfield, and A. B. Stout

The several clones of *Lilium tigrinum* in general cultivation are known to have a somatic number of 36 chromosomes (Takenaka and Nagamatsu, 1930; Mather, 1935). The diploid number characteristic of other species of *Lilium* is 24 (See especially Strasburger, 1882; Guignard, 1884; Coulter, Chamberlain, and Schaffner, 1897; Allen, 1904; Satô, 1932; and Mather, 1935) and recently certain plants to be classed as *Lilium tigrinum* have come from Japan into culture in America which have a somatic number of 24 chromosomes (Stout, 1933). Satô (1932) mentions "wild" plants of *L. tigrinum* which produce seed but does not report that these or the seedlings grown are diploid. The several clones of *Lilium tigrinum* in culture which have a somatic number of 36 chromosomes are presumably auto-triploid and closely related in respect to origin.

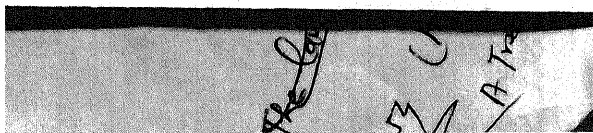
*Plants of the diploid type* are smaller in stature than are the triploids; the flowers are smaller, their segments are narrower, and the spots on the petals and sepals are, as a rule, smaller. The pollen produced is highly viable and the various plants reared from seed are highly productive of seed when there is compatibility either in selfing or in cross-relations.

*The triploid clones* produce few pollen grains that are viable and in addition there is complete incompatibility for all intra-clonal and inter-clonal pollinations. There are then two distinct types of sterility in the members of the species *Lilium tigrinum*: (1) there are physiological incompatibilities in fertilization and (2) there are the abortions of spores which are frequent in the triploid clones.

The studies here reported are especially concerned with the various critical stages in the microsporogenesis of triploids with reference to the abortions which develop. Some comparisons are made with the various corresponding stages of microsporogenesis in diploid plants.

### Material and Methods

Flower buds were collected from both the diploid and the triploid forms of *L. tigrinum*. Temporary aceto-carmin preparations were made from each bud. If the Pollen Mother Cells (P.M.C.) were at



the desired state of meiosis two of the remaining anthers were placed in some killing and fixing solution as Allen's modification of Bouin's, Flemming's medium, Carnoy's, or chrom-acetic. These were run through the paraffin method, embedded, sectioned on a microtome 24-30  $\mu$  thick, and stained with crystal violet, Heidenhain's haematoxylin, Flemming's triple, or Feulgen's. The other two anthers from the flower bud were placed in a solution of 100 c.c. glacial acetic + 200 c.c. absolute alcohol for 24 hours and then transferred to 80% alcohol in which they were held until time for study was available. Belling's method for making aceto-carmines smears (Belling, 1926) proved most satisfactory for all stages except very early prophase and almost mature pollen grains. The earlier stages were more easily studied from the haematoxylin or the crystal violet preparations, while Ehrlich's haematoxylin stained the chromatin contents of microspores and microcytes most readily.

### The Identity of the Individual Chromosomes

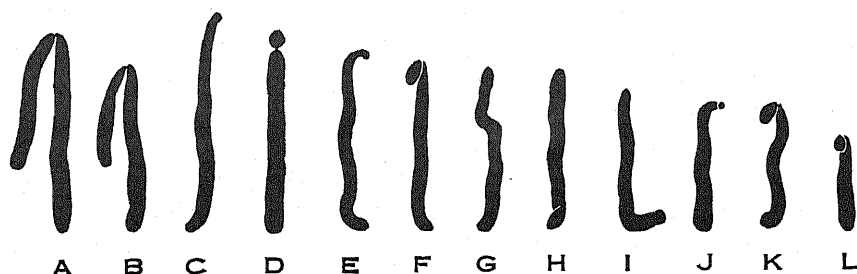
The two sets of 12 chromosomes each in the diploid *Lilium tigrinum* and the three sets of the triploid clones are remarkably alike in respect to the size and the shape of the individual chromosomes. For a triploid type Takenaka and Nagamatsu (1930) figured seven of the twelve chromosomes of a set and made the observation that one of the twelve has a median constriction and one a sub-median while the remainder have terminal or sub-terminal constrictions.

Satô (1932, p. 85) has studied the relative lengths and shapes of the chromosomes of various species of *Lilium* and he concludes as follows:

"The chromosome complement in each species of this genus is composed of elements which bear a striking resemblance in shape to each other, namely four long chromosomes which form two pairs of chromosomes with median and sub-median fiber-attachments, the second class being represented by three pairs of chromosomes with sub-median fiber-attachments, the third by rod-shape with sub-spherical head end, the fourth by the rod-shape with round end."

Satô's figures are of *Lilium tigrinum* clone Flore-pleno and were made from fixed material of somatic mitoses. Our studies were made from aceto-carmines stains of stages in meiosis for triploids which have the single flowers. We found that each of the 12 chromosomes of a set has an individuality in shape, form, and place of fiber attachment which is shown in the following text figure.

The set here shown is from the type clone of the triploid *Lilium tigrinum* which was first sent from China to the Royal Botanic Gardens at Kew, England, by William Kerr in 1804 and propagated since solely by asexual means for horticultural culture. The draw-



Text Fig. 1. Semi-diagrammatic drawings showing relative size and shape of the 12 chromosomes of the triploid set in *Lilium tigrinum*.

ings were made from unusually clear aceto-carmin preparations of the second anaphase, since the chromosomes at this stage are simple and easily studied. A summary of the characteristics of each chromosome is given in the following table:—

Table 1. Tabulated description of the chromosomes of the type clone of the triploid *Lilium tigrinum*.

Chromosome letter	Length in ocular units	Constriction point	Outstanding identifying marks
A	11—7.5	Sub-median	Arm nearly as long as body.
B	9—6	Sub-median	Arm proportionately shorter; ends tend to curve inward; slight undulations.
C	12	Terminal	Long, slender, slight jog in the middle region; no knobs.
D	11	Terminal	Terminal knob; knob never bends over.
E	10	Sub-terminal	Slender, slightly undulate, both ends curved; small knob at polar end.
F	9.5	Sub-terminal	Terminal knob about 2 units in length. Always bent over.
G	9	Terminal	Undulate with definite median jog; no knob.
H	9	Terminal	Simple, straight with posterior end twisted obliquely to the side.
I	8	Terminal	Posterior hook or right angle bend.
J	7	Sub-terminal	Small knob at polar end. Always bent over.
K	7	Sub-terminal	Large terminal knob bent over; body very undulate.
L	5	Sub-terminal	Short with straight body and terminal knob bent over.

Six of the twelve chromosomes, A, B, D, F, G, and K, are easy to recognize and the others are to be identified with a little more

study. In the triploid there is no apparent morphological difference between the homologues, but there are undoubted genetic differences and physiological differences as demonstrated by the irregular pairing of chromosomes and the appearance of univalents in the early stages of meiosis.

The chromosomes of the diploid as well as those of three clones of triploid *Lilium tigrinum*, i.e. splendens, Fortunei, and the intermediate type, were studied with respect to their individuality, but thus far no noteworthy differences between corresponding or homologous chromosomes of the different sets have been observed.

### Meiosis in the Diploid ( $2n=24$ ) *Lilium tigrinum* Compared with That of the Triploid ( $3n=36$ ) Clones

The conspicuous cytological difference between the diploid tiger lily and the triploid type is the presence of the additional twelve chromosomes in the latter. As the stages in microsporogenesis are followed through in both types, it becomes at once apparent that the triploid P.M.C. display very definite irregularities in cytological behavior. Briefly they are as follows:—

- (1) The presence of univalent, bivalent and trivalent spiremes in the prophase as well as the corresponding chromosome associations in the metaphase.
- (2) The distribution of more than 12 chromosomes to daughter nuclei at both I-A and II-A.
- (3) Lagging chromosomes at I-A and II-A.
- (4) Fragmentation of chromosomes and formation of microcysts at I-A and II-A.
- (5) The premature division of univalents at I-A.
- (6) The organization of micronuclei and their degeneration.
- (7) The formation of microcytes and the occurrence of polyspory.
- (8) The abortion of a large percentage of the pollen grains and the ability of only a few grains to function.

In comparing the meiotic stages in diploid and triploid types of *L. tigrinum* we will trace these irregularities from their inception beginning with the post-synaptic phases.

### The Early Stages of Meiosis

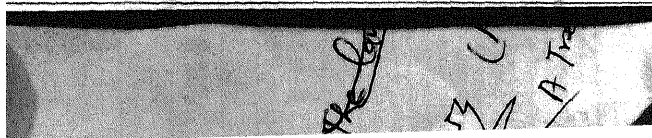
In the diploid type of *Lilium tigrinum* there is complete pairing of homologous chromosomes to the degree that no univalents were observed in these studies (plate 24 figs. 1 and 2). Immediately following synapsis there is a thickening and a shortening of threads and

it is soon evident that there are twelve paired threads of which the free ends are usually to be observed. The chromomeres are of various sizes and distribution along the spireme and they lie side by side in pairs giving a very complete and regular parasynapsis. From early pachyphase it is increasingly evident that a matrix is precipitating around the spireme threads forming a sheath. The sheath gradually acquires chromaticity and forms the bulk of each chromosome. In every nucleus there are one or two nucleoli which persist until late metaphase.

The chromonemata are first evident in the diplophase when they are observed to be twisted about each other. Each chromonema forms the axis of one chromatid (Taylor, 1931; Hsu-siang, 1932). Twisting of the chromatids initiates a coiling of the homologous chromosomes about each other which in this stage reaches its maximum (plate 24 fig. 3). A shortening of the chromatids, and consequently of the chromosomes, gradually takes precedence over coiling so that as diakinesis is approached actual untwisting begins to take place. While coiling is going on, however, there is a tendency for loops and loosely associated regions to appear between twists until shortening takes up the slack. Then the paired chromosomes resemble in appearance a segment of twisted rope until diakinesis brings another opening up of the chromatids. While the chromosomes shorten and commence to uncoil at the end of the diplophase, the chromonemata make no proportional changes. Increased coiling adjusts them to the decreasing length of the chromosome. In this respect the chromatids follow the behavior of the chromosomes rather than that of the chromonemata within. Sub-medial reversal of the direction of coiling of the chromonemata has been noted in both diploid and triploid P.M.C. (plate 24, fig. 3). This is common at the constriction point (plate 24, fig. 8) where the spindle-fiber attachment occurs (Iwata, 1935).

Sass (1934) describes the early prophases as being perfectly normal and implies that synapsis proceeds between pairs of homologous chromosomes. His figures, however, show irregularities in chromosome behavior which indicate that his material was not homogeneous, but was on the other hand derived from several different clones. It is possible that his material may have been collected from both diploid and triploid clones of *Lilium tigrinum* or that sufficient study had not been made of the early prophases to distinguish these irregularities.

In the triploid, irregularities are apparent in the late stages of synapsis in that there are univalent threads as well as bivalent and trivalent associations quite as noted by Horton (1936) in wheat.



The single or univalent spiremes (plate 24, fig. 5) may course independently through the nucleus without contact with other threads and will continue into the later stages as univalent chromosomes.

In the triploid, irregularities in the disposition and association of chromomeres on opposed homologous chromosomes were observed. Corresponding chromomeres on all three spiremes do not always occur and their sizes may be different as noted by Belling (1931). In shape the chromomere aggregates resulting from the conjugation of three homologous chromosomes are somewhat elongated and lobulated (plate 24, fig. 4) in contrast to the diploid in which the paired chromomeres are smaller and transversely lengthened (plate 24, fig. 2). As the prophase proceeds the chromomeres reduce in size and finally disappear, the smallest ones first.

The twisted condition of the three associated chromosomes in the diplophase produces bulky "rope" segments where all three are in conjunction, but the association in most instances and for considerable distances along these chromosomes is loose and irregular. Two spiremes are usually paired while the third may be in contact at one or two points only, and in some cases it may not touch at any point. It then constitutes an unpaired chromosome. Thus there are trivalent, bivalent, and univalent spiremes which originate at synapsis and carry on through diakinesis and metaphase (plate 24, figs. 4 and 5). Shortening of the elements and coiling of the chromonemata proceed normally (plate 24, figs. 3, 6, 8).

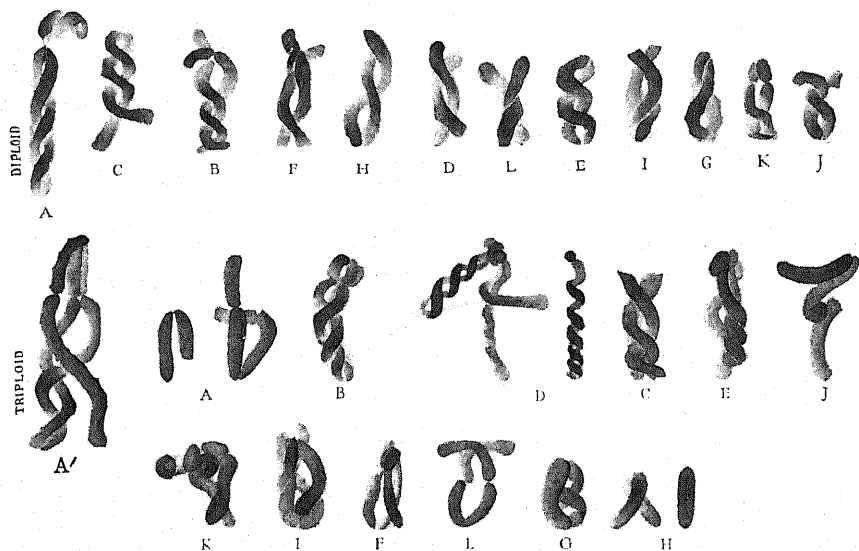
### Diakinesis

In the diploid there are twelve pairs of chromosomes at diakinesis (plate 26, fig. 24). There are no unpaired elements. The chromosomes are shortened and the number of chiasmata in each pair is clearly evident. The individuality of the chromosomes can now be noted in respect to length, number of chiasmata, and the location of the constriction point. The components in each pair are opened out between the chiasmata and this forms characteristic nodes and internodes (plate 24, fig. 6). Each chromosome is composed of two chromatids which apparently contain one chromonema each. The contraction of the chromatids is responsible for progressive decrease in the length of the chromosome. With the decrease in length and the thickening of the chromatids the amount of twisting of the chromatids about each other decreases (Mather, 1935). While the shortening and straightening of the chromosomes is taking place, the chromonema in each chromatid increases its coiling since the chromonemata themselves do not shorten in length (Taylor 1931).

Mather (1935) noted in *Lilium* that chiasmata frequency is



proportional to chromosome length. We corroborate this conclusion from our observations of diakinetid chromosomes in the diploid *Lilium tigrinum*. The greatest number of chiasmata was observed in the pair of A chromosomes in which there were as many as four interstitial and two terminal. Two terminals were observed for E, F, J, and K; none were observed for the pairs of B, D, E, and H. One interstitial was the rule for G, H, I, J, and K, two for D, E, F, and L; three for C; and four for A and B.



Text Fig. 2. Semi-diagrammatic drawings (made to scale) showing configuration of the various chromosome associations at diakinesis in both the diploid and triploid *Lilium tigrinum*.

In the triploid *Lilium tigrinum*, the associations at diakinesis show three main types of irregularities: (1) lack of uniform association between the homologues, (2) chromosomes in different stages of shortening and straightening, and (3) the occurrence of unpaired chromosomes (plate 26, fig. 27). There are irregularities in the number of homologous chromosomes which synapse, and in respect to the reduction in the number of chiasmata and the straightening out of chromosomes the stage seems to be more advanced than in the diploid. Also the individual characteristics of the chromosomes are more evident; for example, the terminal ball on chromosome D is now clearly defined.

In respect to irregularity of association, two of the components are usually in closer contact with each other than with the third (plate 24, fig. 7). In text figure 2 the triploid associations for chromosomes A', B, C, E, G, I, and K illustrate this point. F, L, and J

show about an even degree of association between all three components. In the case of chromosomes A, D, and H the lack of association is evident from the fact that one component of each group frequently remains unpaired. In the unassociated chromosomes (univalents) the shortening and straightening apparently take place more rapidly than in those in which close contact is preserved (B, C, E and L). Definite association of more than three was not observed.

The condition shown for the D chromosome illustrates the behavior frequently seen for unpaired chromosomes. In this case all three of the D homologues are univalents and in each the two constituent chromatids have become not only evident but partially separated. The forces which operate to disjoin associated homologues seems to operate to develop and separate the chromatids of univalents.

### The Association of Chromosomes in the Metaphase of the First Division of Meiosis

*For the diploid.* During the prophases and at the time of the equatorial plate there are 12 pairs of homologous chromosomes. No unpaired chromosomes were observed. Two closely coiled chromonemata may be observed in each chromosome. Twisting of the members of a pair may be seen especially for the pair of the largest chromosomes (A and B) in which case there are points of contact some of which may be true chiasmata (plate 24, fig. 6), or cases of false interlocking. The associations observed at metaphase originate during synapsis. Several cases of interlocking have been noted in this connection (text fig. 2, triploid K) but the twists and coils have undergone reduction until at the time of maximum contraction no more than two twists or one complete coil remain in the longest chromosomes, while the smaller chromosomes have fully straightened out. But at this stage coiling of the chromonemata reaches its maximum in number and compactness. Premature disjunction of chromosomes or the premature separation of chromatids has not been observed for the diploid.

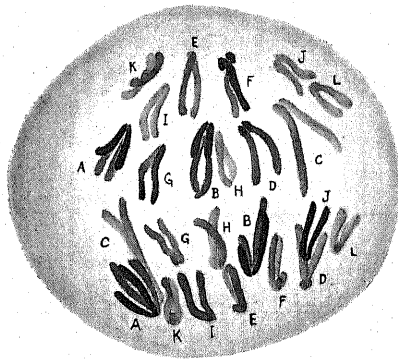
*For the triploid.* Univalents, bivalents, and trivalents are in evidence during the late stages of prophase and in the equatorial plate of the I-M, but it is seldom that all the chromosomes of a triploid P.M.C. are associated in 12 groups of three each. The associations determined for 98 P.M.C. from one anther were as follows:

Number of trivalents . . . .	12	11	10	9	8	7
Frequency . . . . .	2	29	27	25	10	5

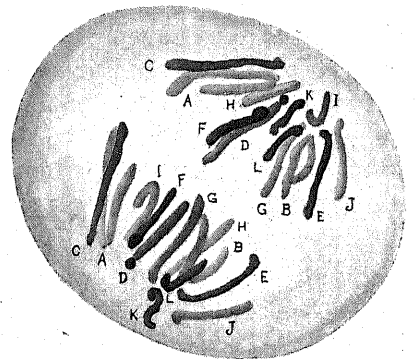
Takenaka and Nagamatsu (1930) reported that in the triploid *L. tigrinum* which they studied the number of trivalents ranged from 6 to 12 with the greatest frequency for 8. Our data indicates that the greatest number of trivalents is much higher than 8.

The associations of the long chromosomes (as A, B, and C) in trivalents present various configurations (text fig. 2, triploid A', A) which are no doubt the effect of differences observed earlier in the synapsis of parts of the threads or the results of chiasmata. The irregularities in the triploid at this stage involve numerical differences in the number of chromosomes which synapse and the configuration of those which are trivalent. In the trivalents there is contact at certain points between any one and the other two, but it is often the case that two are rather closely associated while the third is only partially bound to the others (plate 24, fig. 9). Complete terminalization of chiasmata proceeds up to the beginning of the anaphase but the high viscosity and cohesive qualities of the sheath preserve contact associations beyond that point.

The univalents, bivalents and trivalents are all brought into the level and plane of the equatorial zone (plate 26, fig. 27) and in this particular there are no lagging elements at this stage. The nuclear membrane soon disappears while at the same time the sheath of the chromosome increases in volume and its outer surface becomes more dense. The nucleolus loses its chromaticity at this point and its outline becomes irregular as though parts of it had already undergone partial dissolution. Owing to extreme contraction the individuality of the chromosomes is somewhat obscured.



Text Fig. 3. Semi-diagrammatic drawing made of a diploid P.M.C. of *Lilium tigrinum* at I-A showing the entire set of 24 chromosomes with partially separated chromatids evenly distributed between the two poles. (From an acetocarmine smear preparation).



Text Fig. 4. Semi-diagrammatic drawing made of a diploid daughter cell of *Lilium tigrinum* at II-A showing the entire set of 24 daughter chromosomes (former chromatids) evenly divided between the two poles. (From an acetocarmine smear preparation).

### The Anaphases of the First Division

In the diploid the pairs of homologous chromosomes disjoin with regularity and one set of twelve moves to each pole (plate 26, fig. 25). As the chromosomes proceed, partial separation of chromatids simultaneously takes place so that each of the polar groups is composed of twelve pairs of chromatids. There is no precocious complete separation of chromatids. The chromosomes with terminal and subterminal spindle attachments appear as V-shaped structures (text fig. 3), as shown by Farmer (1895) and later by Allen (1904), with the apex pointed toward a common center, the two chromatids being in contact and bound together only at the point of fiber attachment. The long chromosomes with sub-median attachments approximate double V's with the four ends divergent and the angles coinciding at the constriction region in a bond between each two chromatids. Each chromatid definitely contains now two coiled chromonemata as duplication has taken place. For the entire cell at this stage there are twenty-four pairs of chromatids. There are no lagging chromosomes; and the spindle is bipolar without exception.

In the triploid clones of *Lilium tigrinum* there is in the first division (1) complete disjunction for nearly all members of bivalent and trivalent associations with only an occasional non-disjunction of an homologous pair of chromosomes, (2) partial separation of certain sister chromatids from each other, (3) complete separation of certain of the sister chromatids especially those of the univalents, (4) the lagging of certain chromatin units, and (5) an unequal numerical distribution of chromatin units to the two poles. The rule is that in the anaphases of the triploid types the homologous chromosomes disjoin and also that the two chromatids of each of these chromosomes partially separate, while the chromatids of univalents entirely separate to form the sister chromosomes which would otherwise normally appear in the second division (plate 1, fig. 10).

When they are disjoining, the chromosomes C, D, E, F, G, H, I, J, K, and L pass to the poles as open V's while chromosomes A and B appear as double V's with coinciding vertices and the appearance of these is the same as in the diploid (plate 3, fig. 34). As a rule the unpaired chromosomes (the univalents) fail to leave the equatorial plate and their chromatids completely separate in the equatorial region (plate 3, figs. 28, 29, 30, and 31).

Complete counts were made in various cells with the definite identification of the pairs of attached sister chromatids (diads) and of the separated chromatids (monads) with respect to the distribution in the anaphase of the first division of meiosis. The different distributions observed are given in the following tabulation:—

Table 2. Summary of chromosome distribution at I-A in P.M.C. of triploid *Lilium tigrinum*.

At one pole	Lagging	At second pole	Total equal to
16 diads	10 monads	15 diads	36 diads
17 diads	8 monads	15 diads	36 diads
17 diads	6 monads	16 diads	36 diads
19 diads	4 monads	15 diads	36 diads
17 diads	4 monads	17 diads	36 diads
19 diads	2 monads	16 diads	36 diads
18 diads	2 monads	17 diads	36 diads
18 diads	0 monads	18 diads	36 diads
20 diads	1 diad and 2 monads	14 diads	36 diads

In one of the mother cells listed above one nucleus had 14 chromatin units, the other had 20, and there were 3 lagging, making a total of 37. All the units in the two polar groups were pairs of chromatids (diads). One lagging unit was a V-shaped diad of the chromosome K, but the other two were monads (separated chromatids). Hence the total of 37 is in reality the equivalent of 36 diads. The presence of monads or separated chromatids to the number of 10, as indicated in one case above, gives a total of 41 chromatin units which, however, are equal to the 36 chromosomes of the triploid assembly.

In the case of the distribution of 20 and 14 diad chromosomes noted above, it was determined that at one pole of the 20 there were five pairs of duplicates and of the 14 there were three pairs of duplicates, and in each of these, two homologous chromosomes had passed to the same pole but the pairs were not attached to each other.

The data indicate that the mechanism of distribution operates most effectively for the disjoining of chromosomes and that it tends to separate these into two groups of nearly equal number. Since the number at each pole is nearly always more than 12 it is evident that there is the inclusion of two or more chromosomes of the same type (homologues) within the same polar group. It may, perhaps, be assumed that two of a trivalent group tend to pass to opposite poles and that the third member may go to either. In the counts which were made no case was found in which only 12 chromosomes were found at one pole of the anaphase of the first division, and in no case was 24 found.

The lagging chromatin elements in the early anaphase stages of the first meiotic division in triploids are either (1) pairs of homologous chromosomes, (2) unpaired chromosomes, or (3) single and separated chromatids. The last named are most frequent and numerous. Failure of disjunction sometimes occurs. In this case the pair of homologues may be stretched to form a chromatin band

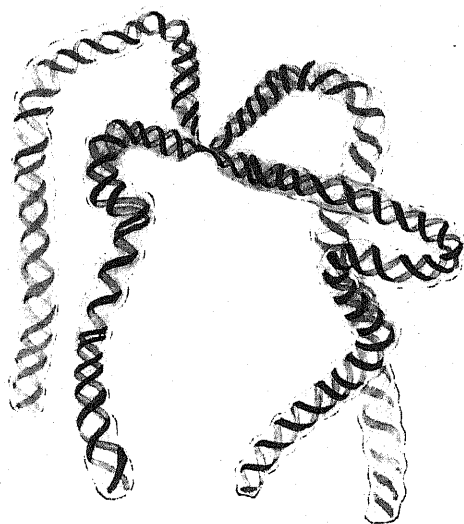
which extends for a considerable distance toward the two poles. If there is a persistent attachment in the distal portion of the two, an "anaphase bridge" (Jensen, 1936) is formed which is later bisected by the formation of a cell plate.

Unpaired chromosomes which lag rarely persist in the equatorial areas as such; that is, with the two chromatids intact in one unit or in partial attachment. It is the rule that the lagging univalents undergo premature separation of chromatids which is the final stage of duplications normally seen in somatic divisions and in the homeotypic divisions of meiosis.

The position and condition of the separated chromatids in the early anaphases indicate certain aspects of the processes which operate. The two sister chromatids separate promptly and completely during the early anaphase and for a time they lie near each other in a somewhat parallel position. They seem to float apart by a repulsion which is operating uniformly at all points including the point of spindle attachment. The separation appears to be largely independent of spindle fiber activity and the operation of such fibers seems to be largely lacking on the separated chromatids in later stages.

Chromosomes A, B, D, E, and K were all identified among the lags but it can not definitely be stated that certain chromosomes of the twelve do not lag. It is, however, certain that the chromosomes which most commonly lag are those for which associations are loose or lacking (as illustrated by chromosome D).

The spindle figures of the first division are bipolar and there is no evidence of the suppression or collapse of the general spindle apparatus which might result in restitution nuclei. All chromatin elements and associations seen in diakinesis are brought into the metaphase plate. From this point on the distribution operates to distribute into two nearly equal groups the chromosomes which have been associated and which have disjoined, but fails thus to operate on



Text Fig. 5. Chromosome A at interphase showing the duplicated chromonemata and the junction of the two chromatids at the constriction point.

those univalents whose chromatids separate prematurely to form sister chromosomes. The only noticeable irregularity in spindle formation is the appearance of the secondary or accessory spindles which form cell-plates to cut off microcytes (plate 25, figs. 21, 22, and 23).

### The Telophase and the Interphase following First Division

The points of special interest in these stages are the organization of the main nucleus in each of the two cells and the behavior and fate of the lagging chromosomes and the fragments of chromatin.

The group of V-shaped pairs of chromatids at each of the two poles (plate 26, fig. 34) is oriented in a manner which suggests the so-called maturation repulsion forces postulated by Takenaka (1933). The individual pairs of chromatids in both diploid and triploid *L. tigrinum* are somewhat elongated during traction toward the pole indicating tension, but as the polar position is reached the shape becomes more undulate. The group of chromosomes, as a rule, is at first star-shaped and somewhat flattened in one plane. But soon the nuclear membrane appears and the plate of chromosomes becomes bent in conformity to its curved surface. No cytoplasm appears to be included within the nuclear space which becomes spherical in shape. Concurrent with the organization of the nuclear membrane the sheath of the chromatids loses volume and practically disappears disclosing thereby the spiral chromonemata. Each chromonema has duplicated and in most of the chromatids the chromonemata have actually separated giving the characteristic criss-cross of the double spiral. In some regions among the chromosomes the chromonemata may still be seen unseparated (text fig. 5). The chromosomes retain their V-organization the ends of the chromatids remaining divergent. They elongate and become tortuous but there is no tendency evident of the free end of one "V" fusing with that of another as suggested by Allen (1904). Lengthening of the chromosomes is evident with the spinning out of the coils (plate 25, fig. 23). No nucleolus is organized and no anastomoses between chromatids are seen.

While the interkinetic nucleus is being organized, the phragmoplast is expanding toward the periphery and any fragments and lagging elements not included with the polar groups at the time the nuclear membrane appears are swept along to the periphery and remain there either to undergo degeneration or else, if in sufficient quantity, to organize as a micronucleus. A small and secondary spindle may arise in connection with the formation of the dividing wall of a microcyte (plate 25, fig. 23). In the diploid no such extruded elements are present. Following the contractions of the next pro-



phase, the chromonemata coil anew and accumulate matrix thus bringing into existence the new chromatids. No chromomeres have been observed at this stage.

*The lagging chromosomes and the fragments of segmented chromosomes* which are a conspicuous feature in the triploids (plate 25, figs. 19 and 20) are, as a rule, left outside of the newly organized nucleus. The fragments, and even entire chromosomes (plate 25, figs. 12-17) soon become rolled up into compact balls and there is increasing opacity, accompanied by the development of one or two lymph vacuoles which are first in contact with the fragment (plate 25, fig. 11 a, b, c, d, e, f, g, h, i). We propose to call the rolled-up chromatin masses which do not become organized as a nucleus or which later appear from disintegrating micronuclei, *microcysts* (plate 26, figs. 32 and 33). Takenaka (1933) calls such bodies "chromatin granules" and Täckholm (1923) calls them "nucleoli" and illustrates them in his figure 33. It appears that the vacuoles separate from the microcysts (plate 25, figs. 11 f, g, h; 18 and 21) and migrate during the interphase toward the nucleus, for cases were observed in which several of them were close to the periphery of the nucleus. The mode of formation, the appearance, and the behavior of these vacuoles suggest that they are formed by the complete dehydration of the chromatin, and that they contain true karyolymph.

Certain chromatids and as many as three chromatids which lag are organized into micronuclei which lie free in the cytoplasm. These may persist but they do not undergo a second division.

*Secondary spindles and microcytes.* At the end of the first meiotic division of the triploid P.M.C. secondary spindles may develop either when cell-plate formation is still in progress or soon thereafter. One pole of such a spindle centers about the lagging chromatin which has become localized in the periphery of the daughter cell following cell-plate formation. The other ends indefinitely in the cytoplasm in the general direction of the nucleus, the fibers converging and disappearing (plate 25, figs. 21, and 22). The cell-plate formed cuts off a dwarf cell termed a microcyte (Hollinghead, 1930) (text figs. 7 b and c, 8; plate 26, figs. 35, 36, 37). These are formed by *secondary spindles* and they originate after the daughter cells have separated. Accessory spindles, on the other hand, occur as an extension of or in conjunction with the phragmoplast during the process of cytokinesis (plate 25, fig. 23). One pole centers about the localized chromatin which may or may not organize a micronucleus, while the other pole is diffuse, some of the spindle fibers proceeding toward one of the nuclei, the others toward the second nucleus. The cell-plate which results appears to be bifurcated



or branched, and the cytoplasm of the microcyte formed comes from the original cell rather than from either of the daughter cells. The chromatin undergoes no movement with either type of spindle, and no evidence of traction or attempted distribution of chromosomes has ever been observed. The sole function of such spindles is cell-plate formation and they may occur in either the first or second divisions of meiosis.

### The Second Meiotic Prophase and Metaphase

Following the brief interphase in which the chromonematal coils spin out to their limit, a second contraction occurs and the chromosomes of both the diploid and the triploid come out of the interphase exactly as they entered it, that is, as diads. At this stage no chromomeres or anastomoses are in evidence.

*In the diploid* there is a second division in each of the two daughter cells and these are simultaneous but independent (plate 26, fig. 26). Four spores of nearly equal size are formed and the primary nucleus of each receives, as a rule, 12 chromosomes. No extruded or lagging chromatin elements were observed in the various seedlings which are to be classed as normal diploids. The chromosomes assembled at each pole are the original chromatids and each one is simple: that is, the limbs of the V's have separated (text fig. 4).

*In the triploid* there is also very uniformly a division for each daughter nucleus and its cell (plate 26, fig. 33). But these nuclei almost always contain more than 12 chromosomes some of which are homologues and also there may be present in the nucleus certain single chromatids. The developments in each of the two main cells involve the separation of the chromatids of those chromosomes which have remained as diads, the distribution of all the chromosomes originating from single chromatids, the organization of these into nuclei or as lagging elements. In addition there are the changes to consider which take place in the micronuclei and microcysts which are included in the two daughter cells from the first division of meiosis.

At the first telophase it is evident from counts that some of the chromatids which constitute the components of equationally divided univalents are included in daughter nuclei. These are to be recognized as monads instead of diads. As the reduction of the matrix proceeds and the extending of the spiral chromonemata continues at interphase the connection between the divergent chromatids can readily be traced. This attachment point can be identified in the diads but in the monads it is absent or less readily observed.

The second division gives, as a rule, from each pollen mother cell four cells of nearly equal size, and in addition there may be formed

at this time smaller cells (microcytes) which may contain lagging chromosomes. These become organized as micronuclei or as microcysts, or they may contain only cytoplasm (plate 26, figs. 35, 36, and 37). The latter are in addition to any microcytes that may form during the first division.

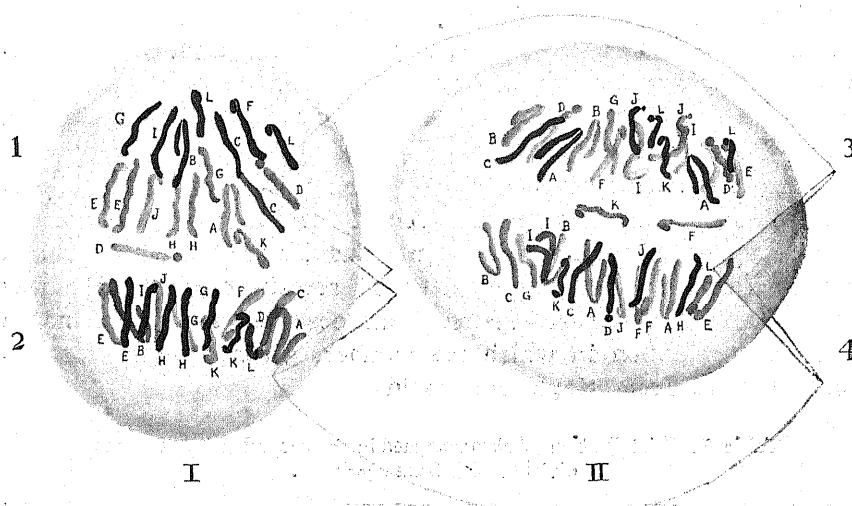
The distribution of chromosomes during the second division was fully determined in 76 cases for each of the daughter nuclei of the four large cells (future pollen grains) and for the lagging elements, and in various cases the identity of certain chromosomes was established. The various distributions observed are given in the following tabulation in which the numbers given refer to chromosomes formed from single chromatids:

Table 3. Distribution of chromosomes in the second or homeotypic division of *Lilium tigrinum*.

In one spindle			In the sister spindle			Total number
One nucleus	Lags	One nucleus	One nucleus	Lags	One nucleus	Chromosomes
17	2	17	17	1	18	72
17	4	15	17	2	17	72
17	1	18	18	0	18	72
17	1	16	18	2	18	72
19	2	17	15	1	15	70
19	0	19	15	1	14	68
18	0	18	16	0	16	68
18	2	16	15	2	14	67

The lagging chromosomes or fragments from the first division are now either rounded up as microcysts, tightly knotted, in advanced stages of disorganization, or disintegrated entirely, or they may be organized in micronuclei. They may be present in these conditions within the larger cells or in the microcytes. In table 3 the number of chromosomes in any one complement of the four large daughter nuclei ranged from 14 to 19. In several cases 72 chromosomes are counted which indicates that each chromosome of each of the three sets had undergone but one equational division even though this occurred in the first division. When the number is less than 72 either some chromosomes did not thus divide or else certain of them were lost through lagging and ultimate disintegration.

The identification of all chromosomes in a late anaphase of the second division was made with respect to the distribution of chromosomes to the four nuclei in a certain P.M.C. of the triploid *L. tigrinum*. In this case there were but three lags evident and these were from the last division. Text figure 6 presents a drawing which shows all the chromosomes and table 4 summarizes their distribution to the two pairs of sister nuclei.



Text Fig. 6. The distribution and identity of all chromosomes of one pollen mother cell of a triploid *Lilium tigrinum* at the anaphase of the second meiotic division.

It is evident that in this case all chromosomes divided once only; that more than a complete set have passed to each of the four nuclei; and that two of the three homologous chromosomes enter either of the two nuclei of a pair by chance distribution to increase the number to more than 12.

Table 4. The distribution of 72 identified chromosomes between the entire four poles in the two daughter cells at II-A of the triploid *Lilium tigrinum*.

														TOTAL
I	Nucleus 1	A	B	CC	D	EE	F	GG	HH	I	J	K	LL	17
	Lags				D									1
II	Nucleus 2	A	B	C	D	EE	F	GG	HH	I	J	KK	L	16
	Nucleus 3	AA	BB	CC	D	E	F	G	H	II	JJ	K	L	17
	Lags						F					K		2
	Nucleus 4	AA	BB	C	DD	E	FF	G	H	II	JJ	K	LL	19
	TOTAL	6A	6B	6C	6D	6E	6F	6G	6H	6I	6J	6K	6L	72

A special study was made of the number of lagging and extended chromatin elements in a large number of cases in which the number of chromosomes in the main nuclei could not be determined. The following table presents the various kinds of distribution of lagging elements observed in each of the two pairs of sister spores and in the additional microcytes. The lagging elements of the first division are given in *italics*, those of the second division are given in the ordinary type. In certain cases the identity of a chromosome was established as indicated in parentheses.

Table 5. Data on lagging chromatin units at the anaphases and telophases of the second meiotic division of the triploid *Lilium tigrinum*.

Lagging in the two pairs of main cells				Data for microcytes		
A	A'	B	B'	NUMBER	NO. OF CHROMATIN UNITS	TOTAL NO. CHROMATIN UNITS
2+1	1+1	1+2 <sup>f*</sup>	1	7	9	18
2+2 <sup>f</sup>	3	4	0	1	2	13
2	3	3+1	2+1	0	0	12
3	1+1	2	3	0	0	10
1+1	1+1	1+2	1	1	1	9
2	4	3	0	0	0	9
3	1	1 <sup>f</sup>	1	2	2	8
1	0	4	2	1	0	7
1(D)	1(B)	1	1+1(C)	1	1(B)	6
2	0	2	2	0	0	6
2 <sup>f</sup>	2 <sup>f</sup>	1	1	0	0	6
1	1(A)	1+1(D)		0	0	5
2	1	0	1(H)	0	0	5
1	1	1	2	1	1	5
1(D)	1(F)	1	1	0	0	3
0	2	1	0	0	0	3
2(D)	1(D)	0	0	0	0	3
0	0	0	0	0	0	0

\* f signifies fragment.

In the groups of cells reported above there was only one tetrad with no lagging chromatin units. The greatest number of such units was 18 in which 7 microcytes were present comprising 9 chromatin elements. The largest number of lagging chromatin elements in any one cell was 4. At this time the lagging units consisted of (1) entire chromosomes of which A, B, C, D, E, F, and H were identified as indicated in the table, and it will be noted these were all lagging from the second division, (2) chromosomes which were rolling up and forming irregular knotted masses, (3) fragments some of which had degenerated into rounded and apparently solid masses, and (4)

chromosomes in micronuclei. Some of each of the last three classes as mentioned were found in microcytes.

### Late Stages of Microsporogenesis

The conditions which exist in the triploid *Lilium tigrinum* at the time of the late stages of microsporogenesis after meiosis is completed and shortly before anthers dehisce may be listed and briefly summarized as follows:—

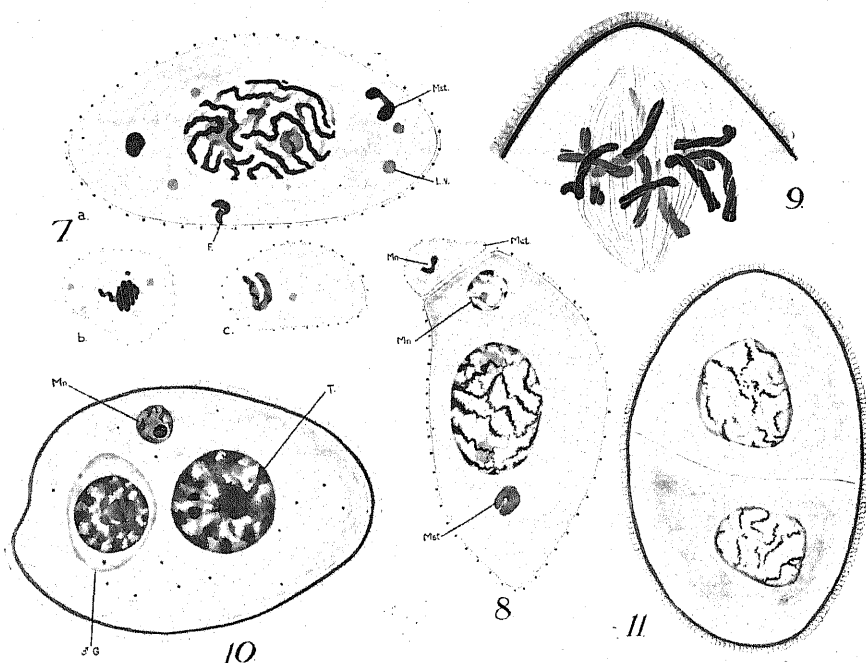
(1) More than four main spores are frequently present, but never less than this number (plate 26, fig. 36). The four sister cells (the potential pollen grains) are rather uniform in size and shape and of larger size than the accessory microcytes (plate 26, fig. 35). There are some differences in size but all contain cytoplasm and one large nucleus, and there is usually present one or more lagging chromatin elements.

(2) As a rule the number of chromosomes in the nucleus of each of the four larger cells derived from a single pollen mother cell is more than 12. The numbers counted at this stage ranges from 14 to 19, but the number of 12 has been found in the equatorial plate of the division of the vegetative nucleus of a microspore (text fig. 9). The organization of nuclei with more than 12 chromosomes seems to be the usual procedure.

(3) In the cytoplasm of the large-sized or primary cells there are (a) degenerating chromosomes or fragments in the form here described as microcysts (text figs. 7 and 8, Mst.), (b) lymph vacuoles which arise in the formation of microcysts (text fig. 7, L.V.), and (c) micronuclei (text figs. 8 and 10, Mn.); and some of each of these may arise in the first division. At least 75% of the mature pollen grains contain one or more microcysts, the largest number observed in any one cell being eight. The degeneration of the lagging or extruded elements of chromatin proceeds both when they lie free in the cytoplasm and when a nuclear membrane is formed.

(4) The micronuclei contain few chromosomes or chromatin fragments. Those which lie in the cytoplasm of a primary cell are reduced to microcysts (plate 26, fig. 38) and hence very few ripe microspores contain micronuclei.

(5) Accessory cells or microcytes are present and conspicuous at this time (text fig. 7, b and c; 8, Mct.). They are much smaller than the primary cells; some of them are formed during the first division, but most of them are formed during the second division. For the most part they are formed after the main cell plates are fully developed. They may contain micronuclei, or microcysts, or both; or there may be no chromatin in evidence.



Text Figs. 7-11. Semi-diagrammatic drawings of stages in the development of the microspores of the triploid. 7, (a). Primary cell containing vegetative nucleus and extruded chromatin material; L. V., lymph vacuoles; Mst., microcysts; and F., chromosome fragment. (b). Microcyte. Chromosome rolling up before forming a micronucleus. (c). Microcyte containing one chromosome and lymph vacuole. 8. Microspore and microcyte drawn in their relative positions. Mn, micronucleus; Mct., microcyte; and Mst., microcyst. 9. Twelve chromosomes at the equatorial plate for the division of the vegetative nucleus. 10. Microspore. G, generative cell; Mn., micronucleus; T, vegetative nucleus. 11. Two vegetative cells in a single microspore.

At the time when anthers of the triploid *Lilium tigrinum* are about to dehisce and discharge pollen there are two rather distinct classes of spores, (a) the large-sized *pollen grains* and (b) the small-sized *microcytes* and these exist in a ratio of about 9 to 1 (plate 26, fig. 39). All these spores contain at least some cytoplasm and have well formed walls. None have collapsed and none appear to be dead.

### The Mature Pollen

During the last few hours before the anthers dehisce and during the early stages of dehiscence while pollen is drying out noteworthy changes occur and obvious degeneration followed by abortion of entire cells appears for the first time. Certain of the large-sized pollen grains do not develop beyond the one-celled stage and these, it is believed, not only do not germinate but abort. It is to be noted

that about 10% of the large-sized pollen becomes empty during the stages of dehiscence (plate 26, fig. 42).

For a considerable number of the large-sized grains the division of the primary cell occurs, but various irregularities in the internal development of pollen grains have been found.

1. The division of the primary cell may result in two cells of nearly equal size (See text fig. 11). This condition was shown by Chamberlain (1897; his figures 19 and 20). The two cells may contain no microcysts or micronuclei and appear as living and quite normal vegetative cells within a common microspore wall.

2. There may be present in the cytoplasm of the pollen grain microcysts and accessory micronuclei in various conditions and most of these arise during meiosis as already described. But in some cases there may be amitosis of the primary nucleus quite as shown by Chamberlain (his figures 13 and 14) which results in small nuclei similar in appearance to the micronuclei formed about lagging chromatin.

3. The generative cell may remain of small size and stay outside of the vegetative cell. It may become separated from the vegetative cell by a wall and may become more or less flattened and aborted (plate 26, fig. 41). This condition was shown by Chamberlain (1897) but the generative cell was interpreted to be a prothallial cell and a micronucleus within the primary cell was interpreted as the generative cell. The structures considered as centrosomes by Chamberlain are, we believe, microcysts in the late stages of degeneration.

4. A large number of the microcytes shrivel into irregular shapes and their contents collapse and disintegrate. Also in the drying of pollen, many of the larger grains become shrivelled to various sizes. Thus the dry pollen, either as such or after it has been placed on sugar-agar media, presents considerable gradation in size, degree of irregularity in shape, and density of contents even for those cells which previously appear to be rather uniform.

The irregularities found in pollen grains may be listed as follows:

1. One vegetative nucleus + one microcyte.
2. " " " + two micronuclei.
3. " " " + four microcysts.
4. " " " + two microcysts + a generative cell.
5. Two " nuclei.
6. " " " + one generative nucleus.
7. Three " "
8. Two " cells in one grain which may or may not be of equal size (text fig. 11).

### The Viability of Pollen

It has been reported (Stout, 1933) that a germination of about 9% was obtained for pollen of *Lilium tigrinum* clone Fortunei which is triploid and that pollen of certain plants of this species which are evidently diploid gave 90% germination. Further tests for germination have given results fully in agreement with this earlier report.<sup>1)</sup>

*Pollen of diploid* in tests for artificial germination on agar (1%)-sugar (5, 10, and 15%) media gave germination for as many as 90% of all grains and only about 1% of all grains was empty and shrivelled. Many of the tubes reached a length of 2000  $\mu$  and some were 3000  $\mu$  in length (plate 26, fig. 43).

*Pollen of the triploid clone Fortunei* was rather fully tested and studied in artificial germination. Microcytes constituted about 10% of all cells to be included as spores and these were empty or nearly empty and gave no germinations (plate 26, fig. 40). There were also mostly empty and shrivelled cells to the number of about 10% which are larger than microcytes and which are certainly tetrad cells whose cytoplasmic contents have died and have largely disintegrated. Careful counts indicated that a germination of nearly 8% of all grains was obtained and the longest tubes observed were 2300  $\mu$  in length. About 70% of all grains did not germinate but did possess cytoplasmic contents either alive or dead which stained conspicuously with aceto-carmine. Tests with aceto-iodine showed the presence of starch. In nearly all cases a vegetative nucleus was observed and in many cases a generative cell was present either inside the vegetative cell or without.

Branscheidt (1930) reports that pollen of *Lilium tigrinum* which does not germinate in sugar culture may be stimulated to a germination of 61% by mixing the pollen with pollen of *Helianthus* and to a germination of 80% by the addition of diastase. The method of culture with diastase used by Branscheidt was employed and also various modifications of it were tested extensively at The New York Botanical Garden for pollen of several types of triploid *Lilium tigrinum*. The increased germination reported by Branscheidt was in no case obtained. Branscheidt does not state what type of *Lilium tigrinum* was studied by him. In the triploid pollen which we have studied at least 20% (including the microcytes) was empty and unable to germinate under any conditions. A considerable portion of the grains which did not germinate had cytoplasmic contents, but in many of these, various abnormalities were observed which together

1) The earlier germination tests were made by Miss Olga Schweitzer and those of later date were made by Mr. Victor Shortino, in the Laboratories of The New York Botanical Garden.



appeared to restrict germination. At any rate in all of our many tests the germination of pollen of triploids has been low (about 8 or 9%) on the same sugar-agar media which give a germination for 90% of the pollen of diploid plants.

Considerable study has been made to determine the internal conditions which may exist in pollen which germinates. Such grains are of the larger size; they possess a thickened wall; their cytoplasm obviously remains alive; there is present a vegetative cell and a generative cell which is within the cytoplasm of the vegetative cell. In certain of the pollen tubes some microcytes and even micronuclei have been observed. The number of chromosomes in the nuclei of germinating pollen can rarely be determined by direct counts and our data on this point are meagre. In one case 12 chromosomes (see text figure 9) were definitely counted during the division of the primary cell, although it cannot be said that the 12 comprised one of each type. It may however, be assumed that microspores whose vegetative and generative nuclei contain a single but complete complement of 12 chromosomes are most likely to function in germination.

### Summary and Conclusion

It is evident that the abortions of the microspores in the triploid clones of *Lilium tigrinum* arise in connection with the presence of an extra set of chromosomes. But the three sets function normally in the somatic tissues of the triploid plants in respect to cell divisions, organizations of nuclei and cells, growth, and differentiation; and they have so functioned in countless numbers of the vegetatively propagated plants of the triploid type over a period of at least 130 years. At the same time in the more intricate processes of meiosis, abnormalities develop and conditions arise which do not occur in the diploid. In the triploid these lead to abortion of pollen grains and the inability of many apparently living grains to germinate.

The abnormalities which can be observed during microsporogenesis appear (1) in various stages of the association or conjugation of the three homologous chromosomes, (2) in the premature separation of the sister chromatids in unpaired chromosomes, (3) in the distribution of chromosomes, (4) in the organization of cells which constitute the complement of spores and (5) in the internal development which produces mature and normal pollen grains.

The association of three spiremies is attempted and in large degree effected for various trivalents in every spore mother cell of the triploid type of *Lilium tigrinum*. Evidently the three homologues for each type of chromosome are able to associate in some degree, but partial associations are frequent and also entire single threads

appear in synapsis, which continue thereafter as univalents. The greater number of chromosomes enter into association as trivalents or bivalents and there is no evidence of larger groups which suggest secondary associations. This with the data that the characteristic chromosome number for species of *Lilium* is  $2n = 24$  and the fact that no  $4n$  species is known seems to indicate clearly that the triploid clones of *L. tigrinum* are autotriploids. The failure of complete association of all spiremes in sets of three is a feature of irregularity which contributes to certain later abnormalities.

The mechanism for the distribution of chromosomes both in the first and in the second division of meiosis is definitely bipolar. Its operation in the first division is to separate the chromosome associations, whether bivalent or trivalent, and to assemble them into two groups of nearly equal number. The only indication of a third group is seen in the lagging chromosomes which first lie in the bipolar spindle: they are left out of the main nuclei and are scattered; and there is no mechanism which can assemble them into a third group or that can operate earlier to place each of the various trivalent groups into three different nuclei. There are, however, accessory spindles which develop feebly as somewhat unipolar figures with one pole centering about lagging chromatin which happens to lie on or near the margin of the plate that is forming. Such spindles operate to produce only microcytes. There is no attempt to assemble or move the several chromatin elements to or from the pole where the nucleus may or may not be organized.

It is believed that the repulsion of the members of bivalent and trivalent associations in the triploid *Lilium tigrinum* is very complete and regular although nondisjunction does sometimes occur. Two homologous chromosomes are frequently included in the same nucleus although they have disjoined. The lagging chromosomes of the first division are chiefly univalents and these undergo an equational division which is premature.

The organization of two daughter cells in the first division and of two pairs of sister cells in the second division proceeds with a remarkable degree of precision and each of these contains a fully organized nucleus which nearly always has more than 12 chromosomes. In addition there may be microcyts and micronuclei in the cytoplasm. The organization of microcytes in both the first and the second divisions occurs as a result of lagging chromatin.

The irregularities in the distribution of chromosomes result in (1) lagging elements of chromatin, (2) the distribution of more than 12 chromosomes to the main nucleus of nearly all large-sized pollen grains, which necessarily involves the presence of one and

often two homologues of certain chromosomes or of two sister chromatids.

The lagging chromosomes contribute (1) to the incomplete distribution of chromatin between the daughter nuclei in both the first and the second divisions, (2) to the premature separation of univalents, (3) to the formation of microcysts and micronuclei many of which disintegrate in the cytoplasm, and (4) to the formation of microcytes.

The abnormal developments which may *actually be observed* within the pollen cells involve the following:—

1. The primary nucleus may fail to divide to form a generative cell or several micronuclei may arise from it by amitosis.

2. The division of the primary cell may result in two cells which may or may not be nearly equal in size (see text figure 11), neither of which can be considered as a generative cell.

3. The generative cell may be produced by a normal mitosis and the typical unequal division of cytoplasm but it may fail to enter the cytoplasm of the vegetative cell, in which case it often shows some disintegration.

4. The generative cell may enter the vegetative cell but fail to divide, or its nuclear material may form into more than two nuclei.

5. The lagging chromatin elements in the cytoplasm undergo noticeable and rapid disintegration. Microcysts form directly or by degeneration of micronuclei and later they disappear.

The conditions which are to be *recognized as directly contributing* to the death and abortion of the various classes of pollen grains as well as to their inability to function are as follows:—

1. A decrease in the total chromatin content which gives less than a normal haploid set of 12. This is especially seen in the micronuclei of microcytes in none of which there are more than two or three chromosomes and usually less. The contents of such cells disintegrate quickly during anthesis and no such cells germinate.

2. An increase of chromosomes in nuclei of large grains above the normal haploid number of 12. The evidence is that an even 12 chromosomes are seldom obtained.

3. Certain of the microspore cells abort and their contents die during anthesis. In these it is certain that one or more of the conditions noted above (1, 2, 3, 4 and 5) may exist and contribute to the abortions. It is observed that in the late stages in the abortion of these cells disintegration of the chromatin content of the nucleus may be observed.

4. Many microspores in which one or more of the final stages of internal development does not occur (1, 2, 3, 4 and 5 above)

appear to be alive although they may not germinate. The cytoplasm and the nucleus of the primary vegetative cell appears to be quite normal and intact.

The abortions of pollen may be attributed to conditions within the nuclei, to conditions in the cytoplasm, or to the combined action of both. Within the nuclei of the microspores there are as a rule more than one set of chromosomes, or at least more than 12, but as far as our observations go it is doubtful if there are ever as many as 24 and we find no evidence that restitution nuclei may form. The chromosome complement may, hence, be unbalanced in number, in genic complement, and in physiological processes. In the cytoplasm the obvious source of possible disturbance is the disintegration of chromatin material. This begins to occur in the telophase of the first division and progresses in degree and extent with the addition of more lags in the second divisions through the last stages of sporogenesis and even into pollen tubes of germinating spores. These disintegrations may, perhaps, be assumed to be toxic or to exert other unfavorable influence on the cytoplasm, on the chromatin within nuclei, or on both, which inhibits proper development of pollen.

Thus in the triploid *Lilium tigrinum* the presence of a third set of chromosomes in a meiotic mechanism which operates to produce two groups of nearly equal number gives irregularities in the mechanical redistribution of these three sets which seldom realize the normal haploid sets. These bring about definite disturbances in the organization of cells and nuclei and in the development of spores. The final abortions and death, as well as certain failures in germination, are to be regarded as internal for each cell or microspore. The organization of the nuclei in respect to kind and number of chromosomes and the disintegration of chromatin in the cytoplasm are to be regarded as the chief active influences.

The abortions of spores in autotriploids, as in *Lilium tigrinum*, in various other unbalanced polyploids, and in many  $F_1$  hybrids constitute a rather definite type of sterility which is distinct from similar abortion in unisexualism, and especially in diploids where definite pollen lethals of individual genetic value may be determined in the behavior of chromosomes. In the *Lilium tigrinum* the intrusion of a third set of homologous chromosomes was a fortuitous and incidental matter. On account of this the mechanism of meiosis which operates for bi-distribution, and the mechanical and physiological activities of microsporogenesis combine to produce the various irregularities which we have herewith described. These operate finally, either singly or in combination, to effect abortion within the cells which comprise the pollen.

## Literature Cited

- Allen, Charles E. 1904. Chromosome Reduction in *Lilium canadense*. Bot. Gaz. 37: 464-471.
- Belling, John. 1926. The Iron-aceto-carmin Method of Fixing and Staining Chromosomes. Biol. Bull. 50: 160-161.
- 1931. Chromomeres of Liliaceous Plants. Univ. Calif. Pub. Bot. 16: 153-170.
- Branscheidt, P. 1930. Zur Physiologie der Pollenkeimung und ihrer experimentellen Beeinflussung. Planta 11: 368-456.
- Coulter, John M., Chamberlain, C. J., and Schaffner, John H. 1897. Contribution to the Life History of *Lilium Philadelphicum*. Bot. Gaz. 23: 412-452.
- Farmer, J. B. 1895. Über Kerntheilung in *Lilium*-Antheren besonders in Bezug auf die Centrosomen-Frage. Flora 80: 56-67.
- Guignard, L. 1884. Recherches sur la structure et la division du noyau cellulaire. Ann. Sci. Nat. Bot. Ser. IV, 17: 5-59.
- Hollingshead, Lillian. 1930. Cytological Investigations of Hybrids and Hybrid Derivatives of *Crepis capillaris* and *Crepis tectorum*. Univ. Calif. Pub. Agri. Sci. 6: 55-94.
- Horton, Ethel Sue. 1936. Studies in the Cytology of Wheat and of a Wheat Species Hybrid. Am. Jour. Bot. 23: 121-129.
- Hsu-Siang. 1932. Structure of Somatic Chromosomes in *Lilium tigrinum*. La Cellule 41: 165-178.
- Iwata, Jiro. 1935. Chromosome Structure in *Lilium*. Mem. Coll. Sci. Kyoto Imp. Univ. (ser. B) 10: 275-288.
- Jensen, H. W. 1936. Meiosis in *Rumex*. I. Polyploidy and the Origin of New Species. Cytologia 7: 1-22.
- Mather, K. 1935. Meiosis in *Lilium*. Cytologia : 354-380.
- Sass, John E. 1934. Chromosome Fragmentation in *Lilium tigrinum* Ker. Am. Nat. 68: 471-475.
- Satô, M. 1932. Chromosome Studies in *Lilium*. Bot. Mag. (Tokyo) 46: 68-88.
- Stout, A. B. 1933. Sterilities in Lilies (*Lilium*) R.H.S. Lily Year-Book 142-171.
- Strasburger, E. 1882. Über den Teilungsvorgang der Zellkerne und das Verhältnis der Kernteilung zur Zellteilung. Arch. mikrosk. Anat. 21: 476-590.
- Täckholm, Gunnar. 1923. Zytologische Studien über die Gattung *Rosa*. Acta Horti Bergiani 7: 97-381.
- Takenaka, Y. 1933. On the Irregular Meiotic Division in the Pollen Mother Cells of *Lilium tigrinum* Ker-Gawl. Bot. Mag. (Tokyo) 47: 125-131.
- Takenaka, Y. and Nagamatsu, T. 1930. On the Chromosomes of *Lilium tigrinum* Ker-Gawl. Bot. Mag. (Tokyo) 44: 386-391.
- Taylor, Wm. Randolph. 1931. Chromosome Studies on *Gasteria*. III Chromosome Structure during Microsporogenesis and the Post-meiotic Mitosis. Am. Jour. Bot. 18: 367-386.

## Explanation of Plates

## Plates 24 and 25

All figures are concerned with the stages in the microsporogenesis of the diploid and triploid *Lilium tigrinum*.

- Fig. 1. Prophase of a P.M.C. of the diploid *Lilium tigrinum* showing synapsed spiremes at pachytene. Iron-haematoxylin.  $\times 1600$ .
- Fig. 2. One pair of homologous chromosomes at pachytene removed from the above and enlarged to show the character and pairing of chromomeres. Matrix shown to be present.  $\times 3340$ .
- Fig. 3. A pair of homologous chromosomes from the diploid *Lilium tigrinum* at diplotene showing not only the dual nature of each chromosome but also their manner of coiling and a true chiasma of chromatids. Iron-haematoxylin.  $\times 3340$ .

- Fig. 4. A trivalent association of homologous chromosomes from the P.M.C. of the triploid *Lilium tigrinum* at pachytene showing the failure of one of the spiremes at certain points to join the other two. Iron-haematoxylin.  $\times 3340$ .
- Fig. 5. Segments of bivalent and univalent spiremes from the triploid at pachytene. Iron-haematoxylin.  $\times 3340$ .
- Fig. 6. A pair of homologous chromosomes from the diploid at diakinesis showing thickening and shortening of chromatids, the lessening of the number of coils, and the terminalization of chiasmata. Aceto-carmin.  $\times 3340$ .
- Fig. 7. A trivalent association of three homologous chromosomes from the triploid at diakinesis showing closer association between two of the chromosomes than between either of these and the third. Chiasmata with false interlocking are present. Aceto-carmin.  $\times 3340$ .
- Fig. 8. A loose association of three homologous chromosomes from the triploid at late diplotene showing irregular coiling of the chromatids and the constriction points all separated from each other. C, constriction. Crystal violet.  $\times 2670$ .
- Fig. 9. A trivalent association of chromosomes at late diakinesis of the triploid showing loose association of the third chromosome and the initial stages in the separation of chromatids in each chromosome. Aceto-carmin.  $\times 1900$ .
- Fig. 10. A univalent chromosome from the triploid at metaphase showing that the parallel chromatids with coiled chromonemata are in the early stages of separating from each other. Aceto-carmin.  $\times 1900$ .
- Fig. 11. a, b, c, d, e, f, g, h, and i are drawings of chromatin elements in the triploid showing all stages in the formation of characteristic microcysts (f, g, h) and their ultimate reduction (i). Lymph vacuoles (L. V.) are present. Crystal violet.  $\times 3340$ .
- Figs. 12, 13, 14, 15, 16, 17. Preliminary stages in the formation of micronuclei in the triploid. Figs. 13 and 14 are of chromosome D. Aceto-carmin.  $\times 3340$ .
- Figs. 18 and 19. Types of chromosome fragmentation by the cell-plates in late anaphase of the triploid. Crystal violet.  $\times 2670$ .
- Fig. 20. Fragments of a chromosome segmented by the cell plate rolling up to form microcysts following meiosis in the triploid. Lymph vacuoles are present. Crystal violet.  $\times 2670$ .
- Figs. 21 and 22. Secondary spindles at the end of the heterotypic division in the triploid showing cell-plate formation leading to the cutting off of microcytes. Crystal violet.  $\times 2930$ .
- Fig. 23. Accessory spindles at the end of the homeotypic division of the triploid showing bifurcation of the phragmoplast resulting in the formation of a microcyte cut from the original daughter cell. Iron-haematoxylin.  $\times 1600$ .

#### Plate 26. Photomicrographs

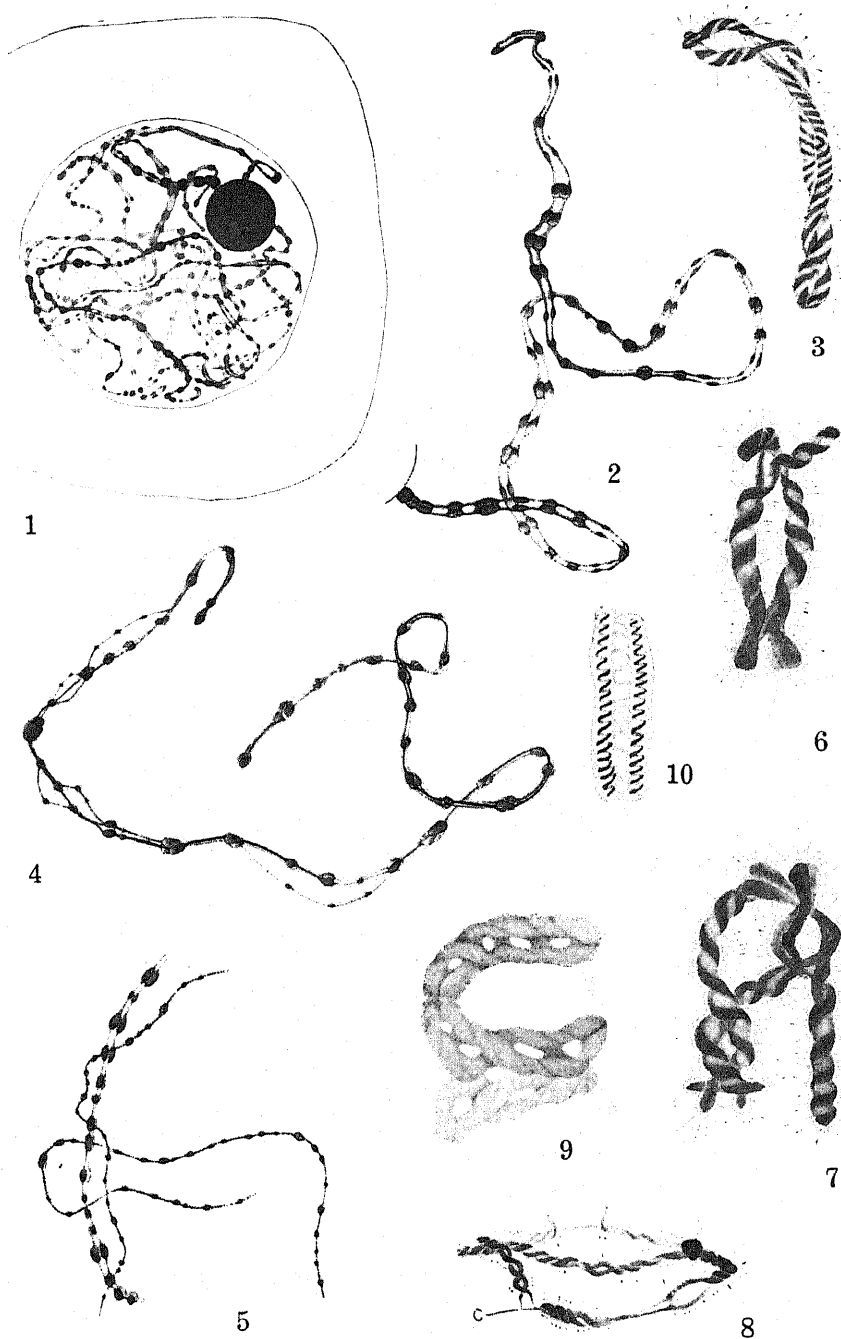
Figs. 24, 25, 26, and 43. Diploid. Figs. 27-42. Triploid.

- Fig. 24. Twelve pairs of homologous chromosomes showing bivalent associations at diakinesis. Both terminal and interstitial chiasmata evident. Aceto-carmin.  $\times 487$ .
- Fig. 25. Equal distribution of chromosomes to each pole at heterotypic anaphase. Aceto-carmin.  $\times 230$ .
- Fig. 26. Equal distribution of chromosomes to the poles at the homeotypic division. Aceto-carmin.  $\times 230$ .
- Fig. 27. Thirty-six chromosomes showing two univalents, two bivalents, and ten trivalents, at diakinesis. Aceto-carmin.  $\times 497$ .
- Fig. 28. Heterotypic anaphase with five chromosomes lagging in equatorial plate region. Aceto-carmin.  $\times 234$ .
- Fig. 29. Heterotypic anaphase. No chromosomes lagging in equatorial region. Aceto-carmin.  $\times 234$ .
- Fig. 30. Heterotypic anaphase. Lagging chromosome segmented by cell plate formation. Aceto-carmin.  $\times 234$ .
- Fig. 31. Late anaphase with five chromosomes lagging at time cell plate begins to form. Aceto-carmin.  $\times 234$ .

- Fig. 32. Homeotypic metaphase. In the left daughter cell appear two microcysts\* formed from lagging chromosomes of the heterotypic division. Aceto-carmin.  $\times 230$ .
- Fig. 33. Homeotypic anaphase with two lagging chromosomes and microcysts\* from the heterotypic division. Aceto-carmin.  $\times 230$ .
- Fig. 34. Heterotypic anaphase in which the chromatids of each chromosome have partially separated. Aceto-carmin.  $\times 607$ .
- Fig. 35. Tetrads with four microspores of average size containing lagging chromosomes from the 2nd division. Microcyte, and microcyst. Aceto-carmin.  $\times 234$ .
- Fig. 36. Tetrad with six microspores and one microcyte. Aceto-carmin.  $\times 234$ .
- Fig. 37. Tetrad showing four microspores and two microcytes, in one of which there is a micronucleus.\* Aceto-carmin.  $\times 234$ .
- Fig. 38. Microspores with microcysts. Aceto-carmin.  $\times 154$ .
- Fig. 39. Microspores and microcytes shortly before dehiscence of anthers. Microcysts still present. Ehrlich's haematoxylin.  $\times 150$ .
- Fig. 40. Mature pollen grains of triploid clone at time of dehiscence showing about 2% germination on artificial medium. Aceto-carmin.  $\times 35$ .
- Fig. 41. Mature pollen grains at time generative cell is formed. Generative cell aborting. Crystal violet.  $\times 150$ .
- Fig. 42. Ripe pollen. Many grains are fully developed and in some the generative cell is formed. Some have grains aborted and are without contents. Heidenhain's haematoxylin.  $\times 80$ .
- Fig. 43. Germination of pollen, diploid form, on artificial medium. Aceto-carmin.  $\times 35$ .

\* Microcyst, Mst; microcyte, Mct; micronucleus, Mn.

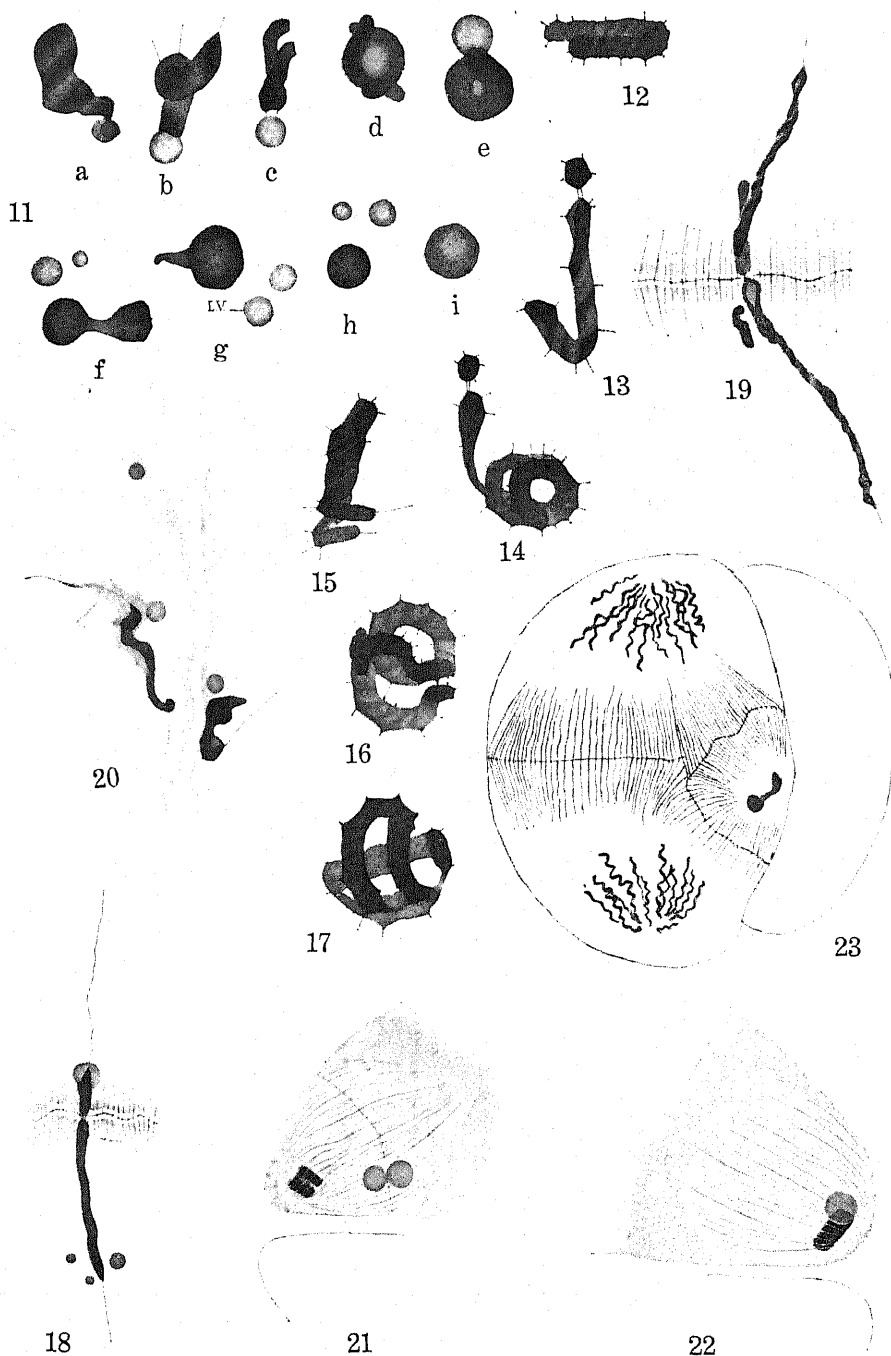




Chandler, Porterfield, and Stout: Microsporogenesis in Diploid and Triploid  
Types of *Lilium tigrinum* with Special References to Abortions

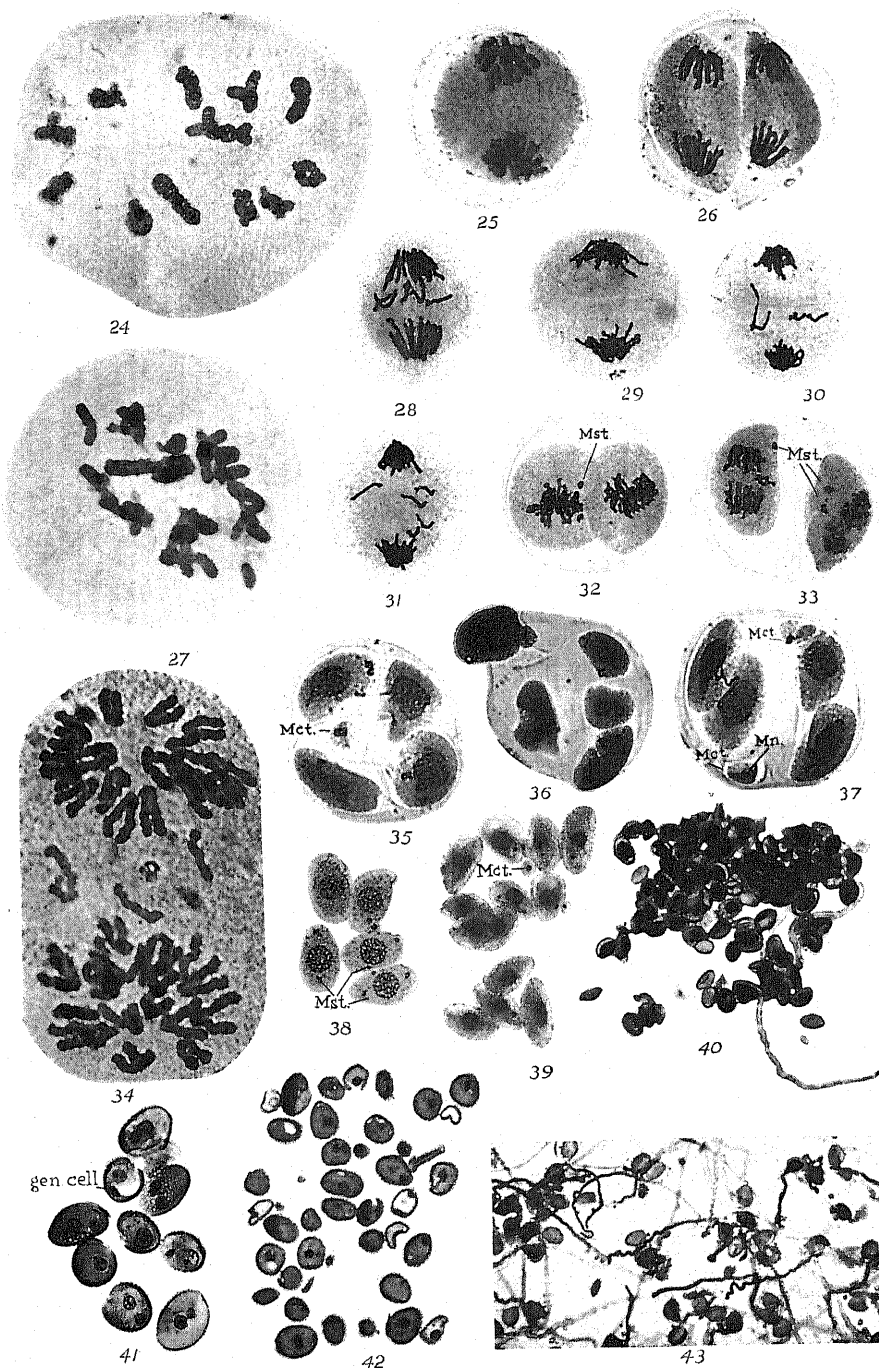






Chandler, Porterfield, and Stout: Microsporogenesis in Diploid and Triploid Types of *Lilium tigrinum* with Special References to Abortions





Chandler, Porterfield, and Stout: Microsporogenesis in Diploid and Triploid Types of *Lilium tigrinum* with Special References to Abortions



**Mikrurgische Untersuchungen lebender Zellen in der Teilung. V.  
Die Einwirkung des Ammonia-Dampfes auf die Mitose bei  
den Staubfadenhaarzellen von *Tradescantia reflexa*<sup>1)</sup>**

Von  
**Bungo Wada**  
Kaiserliche Universität Tokyo  
(Mit 2 Tafeln)

**Einleitung**

Aus den Ergebnissen der III. und IV. Mitteilung dieser Untersuchungen, in welchen die Reduzierungsvorgänge der Chromosomen zur Struktur des Ruhekernes kausalanalytisch verfolgt worden sind, können wir jetzt umgekehrt auf die Entwicklungsvorgänge der Chromosomen aus dem Ruhekerne Folgerungen ziehen. Am Anfang der Mitose werden die chromosomenbildenden Substanzen, namentlich die Chromatinfäden in der Prophase, des Quellungswassers von außen bedürfen. Dann werden die Chromatinfäden anquellen und sich weiter zur Spiralstruktur des Chromosoms aufwinden. Sind die Entwicklungsvorgänge dieser Art, so können wir im Gegensatz zu den Plasmolyse- und Austrocknungsversuchen auf eine andere Behandlung schließen, welche die Chromatinfäden mäßig quellen läßt und die Bildung der Chromosomen beschleunigt. Eine der Substanzen, welche eine solche Wirkung auf die Chromatinfäden ausüben könnte, ist der Ammonia-Dampf.

Was die Wirkung des Ammonia-Dampfes auf die Chromosomen betrifft, so hat KUWADA (1932) nachgewiesen, daß der Ammonia-Dampf die Grundsubstanz des Chromosoms auffallend, das Chromonema selbst dagegen fast kaum quellen läßt. Diese Eigenschaft des Ammonia-Dampfes, für die ich mich hier interessiere, besteht darin, daß die Einwirkung des Ammonia-Dampfes auf die Mitose der Wirkung der Plasmolyse und der der Austrocknung gerade entgegengesetzt erscheint, und ich bemühte mich zu beweisen, daß die Quellung der Chromatinfäden sowie auch die der Chromosomen sich nicht katachromatisch sondern anachromatisch verhält. In der vorliegenden Mitteilung habe ich daher die Einwirkung des Ammonia-Dampfes auf die Mitose in verschiedenen Teilungsstadien hauptsächlich im lebenden Zustande der Zelle und zum Teile durch die Färbung mit Azetokarmin verfolgt.

1) Contributions from the Divisions of Plant-Morphology and of Genetics, Botanical Institute, Faculty of Science, Tokyo Imperial University, No. 183.

### Material und Methode

Als Untersuchungsmaterial brauchte ich die sich teilenden Zellen der jungen Staubfadenhaare von *Tradescantia reflexa* und beobachtete sie in einem hängenden Tropfen von 2% Rohrzuckerlösung in der Feuchtkammer. Mit Hilfe des Mikromanipulators nach PÉTERFI führte ich die Versuche fast in derselben Weise wie bei dem Austrocknungsversuche aus (WADA 1936 b, S. 363).

Beim Ammonia-Versuche stellte ich eine Waschflasche mit Ammoniak zwischen eine Mundpipette und ein Doppelgebläse und verband die beiden mit Gummiröhren. Beim Drücken des Doppelgebläses dringt der Ammonia-Dampf, welcher durch die Luft bedeutend verdünnt worden ist, durch die Mundpipette in die Feuchtkammer ein. Die Menge des in die Feuchtkammer eingeführten Ammonia-Dampfes ist also auffallend gering. Daher färbt sich das Curcuma Probierpapier nur an der die Pipettenöffnung berührenden und an der dem Dampfe ausgesetzten Stelle rötlich und entfärbt sich sofort, wenn der Zufluß des Dampfes aufgehört hat. Eine solche geringe Menge des Ammonia-Dampfes wirkt kaum schädlich auf die Zelltätigkeit ein, jedoch ist sie schon genügend, die Chromosomen lebender Zellen anquellen zu lassen.

Während die Zellen dem Ammonia-Dampfe ausgesetzt waren, beobachtete ich kontinuierlich die Veränderungen der Teilungsfiguren. Natürlich starben die Zellen in einigen Fällen infolge der Aufnahme übermäßiger Menge des Ammonia-Dampfes durch die Schädigung des Protoplasten ab. Die wichtigen Veränderungen einzelner Teilungsfiguren wurden während der Experimente mit Hilfe von LEITZ „Makam“ wiederholt mikrophotographisch aufgenommen.

### Versuch 1. Prophasekern

Von 10 Zellen in der Prophase erhielt ich 4 Fälle, in welchen die Prophasekerne zwar einmal durch die Einwirkung des Ammonia-Dampfes homogen anquollen, aber späterhin die Bildung der Chromosomen ebenso wie auch die der Tochterzellen vollendeten. Die Prophasekerne anderer 5 Zellen starben durch die Aufnahme einer übermäßigen Menge des Dampfes ab. Der letzte befand sich in dem frühesten Zustand der Prophase, sodaß er nach der Behandlung mit dem Dampf zum ruhenden Zustande zurückkehrte. Von den ersten 4 Fällen gebe ich hier einen Fall über das Verhalten der Chromatinfäden in der Prophase zu Protokoll.

25. August 1934. Der Innendurchmesser der Mundpipette ist  $40\mu$ . Der Prophasekern befindet sich in einer Terminalzelle des Haares.

- 8:50<sup>1)</sup> Prophasekern vor dem engen Knäuelstadium. Photographiert (Taf. 27, Fig. 1).
- 8:55 Einsetzen des Zuflusses des Ammonia-Dampfes. Die Chromatinfäden quellen sogleich an und der Kernraum sieht homogen aus. Gleichzeitig stelle ich den Dampfzufluß ein.
- 8:56 Der Kern sieht noch homogen aus. Die Ruhekerne sind jedoch kaum verändert.
- 8:58 Die Kernstruktur tritt wieder schwach im Kernraum auf, wobei sie etwas granulär aussieht. Um diese Zeit erscheint die Quellung des Zytoplasmas, und die Vakuolen kontrahieren sich kugelförmig (Taf. 27, Fig. 2).
- 9:10 Das angequollene Zytoplasma setzt die Entmischung ein. Daher fangen die Vakuolen an, sich zu vergrößern.
- 9:43 Metakinese. Die Bildung der Chromosomen vollendet (Taf. 27, Fig. 3).
- 9:46 Metaphase.
- 11:54 Späte Telophase. Die Zellplatte ist ausgebildet, aber sie sieht noch dünn und schwach aus.
- 15:56 Die Bildung der Tochterzellen ist vollendet. Der Zustand des Zytoplasmas sowie der Scheidewand ist vollständig normal (Taf. 27, Fig. 4).

Vergleicht man das Verhalten des Prophasekernes (Taf. 27, Fig. 2) zu dem Ammonia-Dampfe mit demjenigen des Ruhekernes (Taf. 28, Fig. 14), so bemerkt man, daß die Quellung des Ruhekernes unter der Einwirkung des Ammonia-Dampfes nicht so bedeutend auftritt wie die Quellung des Prophasekernes oder die der Chromosomen selbst. Diese Erscheinungen beweisen, daß die Chromosomengrundsubstanz, welche durch die Einwirkung des Ammonia-Dampfes leicht anquillt, nur im sich in der Teilung befindenden Kern vorhanden ist. Daher schließen sich diese Ergebnisse in Bezug auf die Entstehung der Chromosomengrundsubstanz an die Auffassung von KUWADA und NAKAMURA (1934a S. 356) an, indem die Matrix der Chromosomen sich *de novo* in der Prophase differenziert.

Erst durch die übermäßige Aufnahme des Ammonia-Dampfes quillt der Ruhekern auch ein wenig an und sieht homogen aus (Taf. 28, Fig. 16). Im Gegensatz zur reversiblen Homogenisierung des Prophasekernes ruft der homogenisierte Ruhekern weiter die Entmischung seiner selbst hervor und stirbt durch die Koagulation ab.

## Versuch 2. Quellung der Chromosomen

Von 20 Teilungsfiguren in der Metakinese und in den nachfolgenden Teilungsstadien, die mit dem Ammonia-Dampfe behandelt wurden, vollendeten 10 Zellen nach vorübergehender Quellung der Chromosomen die Bildung der Tochterzellen fast in normaler Weise. 8 Zellen starben dabei durch die Koagulation ab. Die anderen 2 Zellen bildeten dem Syndiploidkerne ähnliche Chromosomenklumpen. Von den ersten 10 Zellen gebe ich hier einen Fall zu Protokoll.

1) 8:50 bedeutet 8 Uhr 50 Minuten; die Worte Uhr und Minute sind (wie auch in den folgenden Zeitangaben) ausgelassen.



25. August 1934. Der Innendurchmesser der Mundpipette ist  $40\mu$ . Die Mitose befindet sich in einer Terminalzelle des Haares.
- 13:30 Metakinese. Photographiert (Taf. 27, Fig. 5).
- 13:35 Metaphase. Durch einen schwachen Zufluß des Ammonia-Dampfes quellen die Chromosomen sofort an und sehen homogen aus. Jedoch behalten die Spindelsubstanzen dabei ihre spindelförmige Gestalt bei, ohne daß sie irgend welche sichtbare Veränderung zeigen.
- 13:37 Das Zytoplasma quillt jetzt etwas an, gleichzeitig tritt in den sich kontrahierenden Vakuolen eine Anzahl von tropfenförmigen Fällungen auf, welche die BROWNSche Bewegung fortsetzen (Taf. 27, Fig. 6).
- 13:55 Die Entmischung des angequollenen Zytoplasmas findet statt, und die Zelle fängt an, sich zu erholen. Dazwischen kehren die Chromosomen im Spindelraum zu ihrem normalen Zustande zurück, wobei die Längerspaltung einzelner Chromosomen sichtbar wird. Dagegen verschwinden in dieser Zeit die Fällungen in den Vakuolen nach und nach.
- 15:36 Späte Anaphase. Durch die Entmischung des Zytoplasmas vergrößern sich die Vakuolen beträchtlich, wobei die Polplasmastränge<sup>1)</sup> und andere Plasmastränge in den Vakuolen verschwinden und die gestörten Zytoplasmagliederungen die Tochterchromosomenwanderung zum Spindelpol auffallend verzögern. Zum Beispiel dringt der eine der Spindelpole (der untere Pol in Fig. 7) ohne Polplasmastränge in die Vakuole ein (Taf. 27, Fig. 7).
- 15:43 Der Spindelpol in der Vakuole bewegt sich entlang der einen der Seitenwände, und die Teilungsfigur tritt nunmehr in die mittlere Telophase ein (Vgl. WADA 1935, Taf. 14, Fig. 2 u. 3).
- 16:12 Die Tochterkern- und die Scheidewandanlage sind normal ausgebildet, und des Zellteilung ist ebenfalls vollendet (Taf. 27, Fig. 8).

Auf Grund von Lebendbeobachtungen können die Einwirkungen des Ammonia-Dampfes auf die Teilungsfiguren zu den folgenden drei Stufen analysiert werden.

Als erste Stufe der Ammonia-Wirkung bemerkt man, daß die Grundsubstanz der Chromosomen zuerst anquillt und die Quellung des Zytoplasmas ein paar Minuten später einsetzt. Infolge der Quellung der Chromosomengrundsubstanz verschwinden die Zwischenräume einzelner Chromosomen; dann sieht der Spindelraum homogen aus. Beim Quellen des Zytoplasmas kontrahieren sich die Vakuolen deutlich und werden kugelförmig. In diesen kontrahierenden Vakuolen treten tropfenförmige Fällungen auf und setzen die BROWNSche Bewegung fort. In dieser ersten Stufe der Ammonia-Wirkung erleiden das Chromonema, die Spindelsubstanzen und der Phragmoplast fast keine sichtbare Veränderungen (Taf. 28, Fig. 14, 19, 20).

Als zweite Stufe der Ammonia-Wirkung setzt die Entmischung der angequollenen Chromosomen und des Zytoplasmas fast gleichzeitig ein. Infolge der Entmischung des Zytoplasmas und der Chro-

1) Siehe WADA 1935, S. 396.

mosomen nehmen die Volumen der Vakuolen nach und nach zu und überschreiten schließlich ihre Größe vor der Behandlung. Gegen dieselbe Zeit verschwinden die Fällungen, welche bei der Vakuolenkontraktion im Zellsafte entstanden, allmählich. Das Auftreten und das Verschwinden der Fällungen in den Vakuolen findet reversibel statt.

Während dieser zweiten Stufe der Ammonia-Wirkung behalten das Chromonema, die Spindelsubstanz und der Phragmoplast fast ihren normalen Zustand bei, obwohl die Gestalt des Spindelraumes nicht selten durch die Vergrößerung der Vakuolen deformiert wird. Wenn die sich teilenden Zellen den Wirkungen des Ammonia-Dampfes in der ersten und zweiten Stufe widerstehen können, so erholen sich die Teilungsfiguren, sowie auch die Zelle als Ganzes zu ihrem normalen Zustande, und die Zelle vollendet schließlich die Ausbildung der Tochterzellen.

Wenn die Teilungsfigur, ohne daß sie sich dabei erholt, weitere Veränderungen erleidet, bezeichne ich solche Veränderungen als dritte Stufe der Ammonia-Wirkung; und sie erweisen sich als Degenerationerscheinung. Aus den wiederholten Experimenten bei den Staubfadenhaarzellen von *Tradescantia* konnte ich feststellen, daß der Syndiploidkern, die zweikernige Zelle und dgl., welche bei den Plasmolyse- und Austrocknungsversuchen experimentell hervorgerufen werden können, durch die Einwirkung des Ammonia-Dampfes nicht im lebensfähigen, sondern lediglich im degenerierten Zustande der Zelle entstehen.

### Versuch 3. Der Klumpen der Chromosomen und Azetokarminfärbung

In diesem Abschnitte wende ich mich zur Frage des Chromosomenaggregates, welches entstehen kann, wenn die Mitose übermäßigem Ammonia-Dampf ausgesetzt wird. Am Anfang der Aggregation der Chromosomen gehen bei der Teilungsfigur auch die erste und die zweite Stufe der Veränderungen des Protoplasten vor sich, wie sie in den vorigen Versuchen geschildert sind. Dann treten die Teilungsfigur und das Zytoplasma nunmehr durch weitere Entmischung des Protoplasten in die dritte Stufe der Ammonia-Wirkung ein.

In der dritten Stufe schreiten die Entmischung der angequollenen Chromosomen sowie auch die der Zytoplasmabestandteile, die Veränderungen der Spindelsubstanzen und die des Phragmoplasten meistens auffallend schnell fort. Bei solcher Entmischung vergrößern sich die Vakuolen auffallend, und alle Plasmastränge verschwinden darin. Dann wird die Teilungsfigur entweder zu einer

Ecke der Zelle oder zur Stelle der Zellplatte geschoben, und die Chromosomen treffen dabei zu einem Klumpen zusammen. Der Menge des Ammonia-Dampfes, welche auf die Zelle einwirkt, sowie auch dem Zustande der mitotischen Figuren entsprechend, sind die Veränderungen der Teilungsfigur zeitlich sowie auch morphologisch sehr verschieden. Als Aggregat der Chromosomen bilden die Teilungsfiguren in der Meta- und Anaphase einen homogenen blassen Klumpen (Taf. 28, Fig. 15), und in den noch späteren Teilungsstadien verändern sich die mitotischen Figuren zu einem amitose-ähnlichen Chromosomenklumpen oder zu zwei Chromosomenklumpen in einer Zelle.

Die Zellen, welche solche Klumpen der Teilungsfigur zeigen, sterben durch die Koagulation meistens binnen einiger Stunden ab, jedoch gehen einige erst ein paar Tage später zu Grunde. Hier gebe ich einen Fall zu Protokoll, in welchem der Chromosomenklumpen als Syndiploidkern entstanden ist.

20. August 1934. Der Innendurchmesser der Mundpipette ist  $39\mu$ . Die Teilungsfigur befindet sich in einer Terminalzelle des Haares.
- 11:24 Mittlere Anaphase. Photographiert (Taf. 27, Fig. 9).
- 11:30 Einsetzen des Zuflusses des Ammonia-Dampfes. Die Chromosomen quellen sofort an, und der Spindelraum sieht homogen aus. Fast gleichzeitig setzt die Quellung des Zytoplasmas ein, und die Vakuolen, in welchen eine Anzahl sich bewegender Fällungen auftritt, kontrahieren sich kugelförmig (Taf. 27, Fig. 10).
- 12:08 Die Chromosomen und das Zytoplasma entmischen sich äußerst langsam, wobei die Struktur der Chromonemata einzelner Chromosomen nach und nach hervortritt.
- 12:58 Infolge der Entmischung treffen die beiden Tochterchromosomengruppen zu einem Klumpen zusammen, und seine Struktur ähnelt der eines Syndiploidkernes (Taf. 27, Fig. 11).
21. August. Dieser Syndiploidkern sowie auch der Protoplast erholen sich zum ruhenden Zustande der Zelle, aber die Zelle sieht als Ganzes stark altersschwach aus (Taf. 27, Fig. 12).
22. August. Die Zelle ist durch die Koagulation des Protoplasten abgestorben.

Im Gegensatz zum Syndiploidkern bei den Plasmolyse- und Austrocknungsversuchen gelangte der Chromosomenklumpen bei diesem Experimente schließlich nicht zur vollkommenen Struktur des Ruhekernes (siehe WADA 1936a u. 1936b). In der Tat starb dieser dem Syndiploidkern ähnliche Chromosomenklumpen zwei Tage nach der Behandlung durch die Koagulation des Protoplasten ab.

Bei der koagulierenden Teilungsfigur beobachtete ich auch eine andere interessante Erscheinung. Wenn man eine entmischende Teilungsfigur, welche durch die Wirkung des übermäßigen Ammonia-Dampfes hervorgerufen ist, nochmals dem Ammonia-Dampf einige Minuten lang aussetzt, so fängt diese Teilungsfigur wieder anzu-

quellen an. Dann quellen nicht nur die Chromosomen, sondern auch die Zytoplasmabestandteile so auffallend an, daß die Zelle von ihnen fast vollständig eingenommen wird und kaum Vakuolen zurückläßt (Taf. 28, Fig. 16).

Hier müssen wir darauf aufmerksam machen, daß diese zweite Quellung der Zellbestandteile in keiner Beziehung zu der Vitalität der Zelle steht. Sie findet lediglich als eine postmortale Erscheinung statt. Im Gegensatz zur zweiten Quellung ist die Zelle bei der ersten Quellung noch lebend und bemüht sich dabei, sich zu ihrem normalen Zustande zu erholen, wie es in den Versuchen 1 und 2 geschildert ist.

Um die strukturelle Veränderung der angequollenen Chromosomen zu konstatieren, färbte ich einige Teilungsfiguren nach der Ammoniabehandlung mit Azetokarmin, wobei sich die eine der Teilungsfiguren in dem ersten und die andere in dem zweiten Quellungszustande der Zelle befand. Im Zustande der ersten Quellung können die Chromonemata einzelner Chromosomen verhältnismäßig leicht verfolgt werden (Taf. 28, Fig. 20). Dagegen tritt im Zustande der zweiten Quellung der Zelle eine Anzahl von granulär aussehenden Chromonema-Reihen hervor, und diese Reihen ordnen sich einigermaßen den früheren Anordnungen der Chromosomen entsprechend an, wobei sich die Spiralwindungen der Chromonemata fast vollständig auffasern. Daher erscheint ihre Struktur fast gleichartig wie die des Ruhekernes. Nur ist die Verteilung der aufgefasernten Chromonemata nicht so dicht wie im intakten Ruhekern (Taf. 28, Fig. 17). Die Struktur sowie auch die Entstehungsweise dieser angequollenen Chromosomenklumpen stimmen mit den „imitation nuclei“ von KUWADA und NAKAMURA (1934a) bei den Pollenmutterzellen von *Tradescantia* überein, daher wird dieser Chromosomenklumpen auch von mir für einen „imitation nucleus“ gehalten, welcher aus den Chromosomen der somatischen Zellen entstanden ist.

### Schluß

Aus den Ergebnissen dieser Versuche kann man schließen, daß durch die Einwirkung des Ammonia-Dampfes zuerst die Grundsubstanz der Chromosomen und dann die Zytoplasmabestandteile quellen. Weiter ist festgestellt, daß die mäßige Wirkung des Ammonia-Dampfes die Chromatinfäden in dem Prophasekerne quellen läßt und die angequollenen Chromatinfäden sich zu Chromosomen entwickeln. Diese Tatsache bekräftigt die Hypothese in der zweiten Mitteilung dieser Abhandlung (WADA 1935, S. 395), daß die Chromatinfäden bei ihrer Entwicklung zu Chromosomen des Quellungswassers, bzw. der Quellung derselben, unbedingt bedürfen. Mit anderen Worten

gesagt hängt die Quellung der Chromatinfäden in der Prophase physiologisch mit dem Mechanismus der Chromosomenentwicklung zusammen.

Die Einwirkung des Ammonia-Dampfes bleibt dabei nicht nur bei der Quellung der Grundsubstanzen des Chromosoms stehen, sondern scheint sich mir nach und nach auch auf die Substanzen des Chromonemas auszudehnen und ihnen Veranlassung zur Aufnahme des Wassers zu geben. Daher quellen die Chromonemata selbst auch an und entwickeln sich zusammen mit den Grundsubstanzen zu ausgebildeten Chromosomen (vgl. KUWADA u. NAKAMURA 1934b). Bei der Einwirkung des Ammonia-Dampfes verzögern sich die Fortgänge der Mitose durch die Quellung der Zytoplasmabestandteile in verschiedenen Graden, da die Zytoplasmaarchitektur in der sich teilenden Zelle dabei gestört wird und die Zeit verläuft, bevor diese Störung sich zum die Teilung weiter ausführenden Zustande erholt.

Die Quellung des Prophasekernes findet auch in einer hypotonischen Lösung statt. Ich beobachtete eine Zelle in der Prophase von *Tradescantia*-Haarzellen im Leitungswasser, wobei der Prophasekern zuerst anquoll und die Bildung der Chromosomen, sowie auch die der Tochterzellen, vollendete. Jedoch verzögerte sich die Vollendung der Mitose auffallend, da der Zustand der Zytoplasmabestandteile in der hypotonischen Lösung bedeutend schlechter geworden war.

Auf jeden Fall schließt sich die Entwicklung der Chromatinfäden zu Chromosomen an die Aufnahme des Quellungswassers an. Was die Herkunft des Quellungswassers in der Prophase anbetrifft, vermute ich, daß das Wasser hauptsächlich von dem Dispersionsmittel der Karyolymphe geliefert wurde.

Bei den Anstichversuchen der Prophasekerne beobachtete ich einen Ausnahmefall, in welchem sich der Prophasekern bei einer angestochenen Zelle nach der Quellung nicht zum Chromosomen, sondern zum Ruhekern veränderte (WADA 1932, S. 118). Die Einwirkung des Anstiches auf den Protoplasten kann keineswegs einfach aufgefaßt werden, da nicht nur durch den Anstich in der Zelle einige physikalische Veränderungen hervorgerufen werden, sondern es auch unvermeidlich ist, daß während und nach dem Anstiche einige Bestandteile ihres Inhaltes verloren gehen. Gleichzeitig wird durch die Verminderung des Zellurgors und auch durch die Veränderung der Zellpermeabilität das Gleichgewicht zwischen den Zellbestandteilen gestört. Nunmehr wird angenommen, daß sich der angestochene Prophasekern leichter zum Zustande des Ruhekernes reduzieren würde, als die Zelle sich durch das Ersetzen des Verlorenen zu einem neuen Gleichgewichtszustand der prophasischen Zelle erholen könnte.

Daher kehren die angequollenen Prophasekerne bei der angestochenen Zelle zum Ruhezustande des Kernes zurück.

Im Gegensatz zu diesen Experimenten erhielten ROSENFELD (1933) bei den tierischen Zellen unter der Einwirkung des Ammonia-Dampfes im lebenden Zustande der Zelle verschiedene Modifikationen der mitotischen Figuren, namentlich Syndiploidkern, zweikernige Zelle u. dgl. Die Schwierigkeit des Erhaltens der modifizierten Mitose bei den lebenden pflanzlichen Zellen scheint mir nicht auf der Verschiedenheit der Eigenschaften der mitotischen Apparate, sondern auf derjenigen der Zytoplasmazustände der beiden Lebewesen zu beruhen, da die sich teilenden Zellen von Staubfadenhaaren von *Tradescantia* eben einige Vakuolen enthält und ihr Zytoplasma durch die Ammonia-Wirkung leicht quellbar und bzw. entmischbar ist. Diese Eigenschaften des Zytoplasmas wirken auf die mitotische Figur schädlich ein und machen es unmöglich, daß das Aggregat der Chromosomen sich zum Ruhezustande des Kernes erholt.

In seiner Beschreibung hat ROSENFELD auf die Quellung der Chromosomen nicht verwiesen. Vielleicht war sein Material zu klein, um die Quellung der Chromosomen im lebenden Zustande der Zelle zu konstatieren.

Das andere wichtige Ergebnis dieser Versuche ist, daß unter der Einwirkung des Ammonia-Dampfes die Chromosomen und das Zytoplasma leicht, die Spindelsubstanzen dagegen schwer quellbar sind. Die Quellbarkeit der Chromosomen und des Zytoplasmas tritt durch die Wirkung des Ammonia-Dampfes zweimal hervor, und es ist experimentell festgestellt, daß die erste Quellung im lebenden, die zweite dagegen im postmortalen Zustande der Zelle stattfindet. Weiter konstatierte ich, diesen zwei angequollenen Zuständen entsprechend, zwei Typen der Auffaserungszustände der Spiralwindungen der Chromonemata. Die ähnlichen Beziehungen zwischen der Quellung der Chromosomen und der Auffaserung der Spiralwindungen der Chromonemata haben KUWADA und NAKAMURA (1934a) bereits durch die Wirkung des Ammonia-Dampfes bei den Pollenmutterzellen von *Tradescantia* festgestellt.

Im Gegensatz zur chromatischen Figur zeigt die achromatische Figur unter der Einwirkung des reinen Ammonia-Dampfes fast weder Quellung noch Kontraktion. Daher erleidet die Teilungsfigur unter der mäßigen Wirkung des Ammonia-Dampfes keine Störung der Spindelfigur und vollendet nach einer vorübergehender Quellung der Chromosomen die Karyo- und Zytokinese vollständig. Wenn die Ammonia-Wirkung durch die Mitwirkung irgend welcher Substanzen die achromatische Figur quellen lassen könnte, so würden damit verschiedene Modifikationen der mitotischen Figur, wie der Syndiploid-

kern, die Teilungsumkehr u. dgl., experimentell im lebenden Zustande der Zelle hervorgerufen werden (vgl. WADA 1935, S. 391). Über die Zusammenwirkung des Ammonia-Dampfes mit anderen ätherischen Substanzen will ich in der Fortsetzung dieser Untersuchungen handeln.

### Zusammenfassung

1. Mit Hilfe des Mikromanipulators wurde die Einwirkung des Ammonia-Dampfes auf die sich teilenden Zellen von Staubfadenhaar von *Tradescantia reflexa* hauptsächlich im lebenden und zum Teil im gefärbten Zustande der Zelle untersucht.

2. Wenn die Teilungsfigur einer sehr geringen Menge des Ammonia-Dampfes ausgesetzt wird, quillt zuerst die Grundsubstanz der Chromosomen und dann das Zytoplasma an. Die Chromonemata, die Spindelsubstanz und der Phragmoplast zeigen wenigstens am Anfang der Behandlung eine Weile fast keine sichtbaren Veränderungen.

3. Durch die Wirkung einer mäßigen Menge des Ammonia-Dampfes wird die Quellung der Chromatinfäden und der Chromosomen, jedoch keine Teilungsumkehr hervorgerufen. Die angequollenen Chromatinfäden in der Prophase entwickeln sich zu Chromosomen und vollenden schließlich die Bildung der Tochterkerne.

4. Bei der Wirkung einer übermäßigen Menge des Ammonia-Dampfes entsteht zuerst die Quellung der Chromosomen und des Zytoplasmas. Dann folgt ihr die Entmischung nach. Infolge der Entmischung stirbt die Zelle schließlich durch die Koagulation des Protoplasten ab, wobei die Teilungsfigur als Degenerationserscheinung ein anomales Verhalten zeigt.

5. Diese koagulierende Teilungsfigur quillt nochmals bei weiterer Behandlung mit Ammonia-Dampf auffallend an.

6. Beim Quellen des Zytoplasmas tritt eine Anzahl sich bewegend tropfenförmiger Fällungen in den Vakuolen auf, aber sie verschwinden beim Entmischen des Zytoplasmas gänzlich.

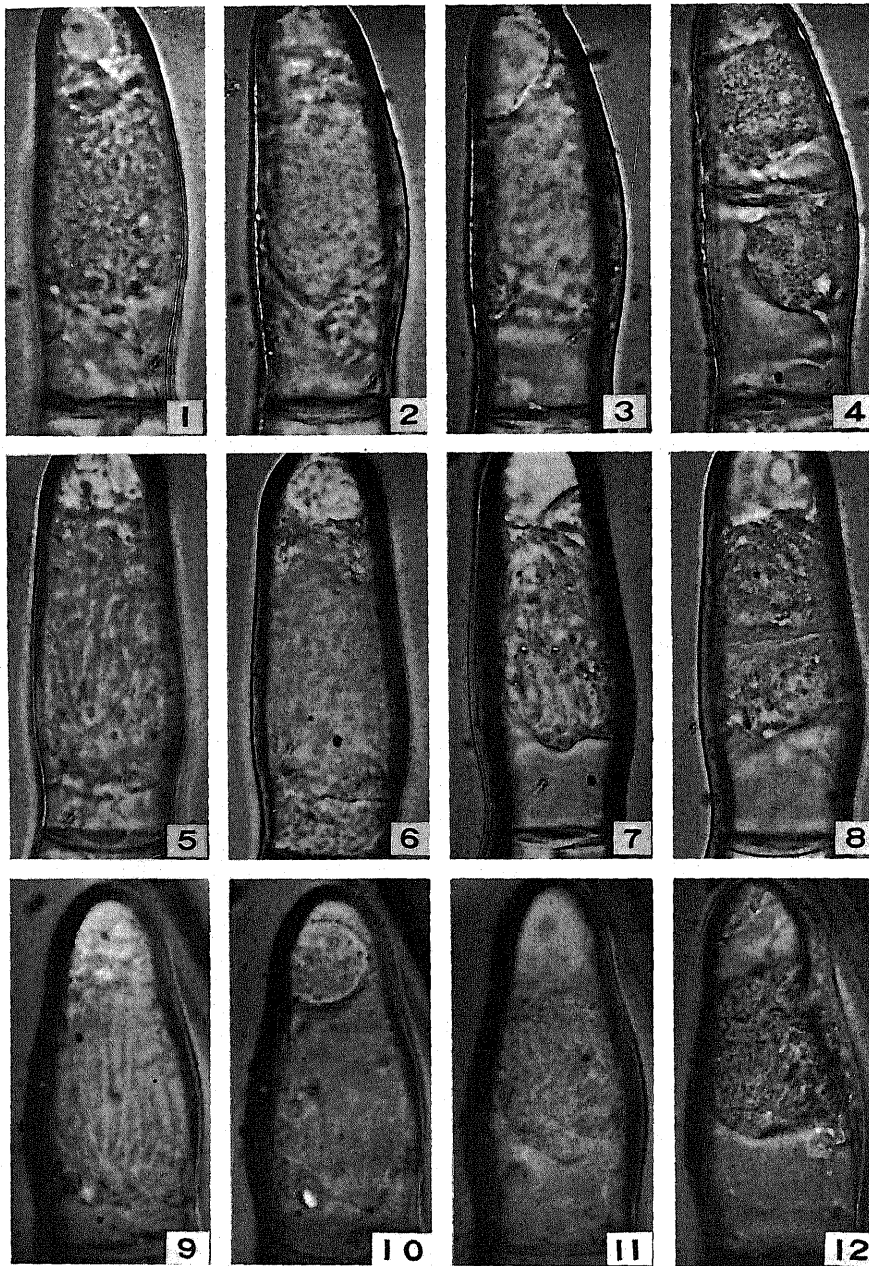
September 1936

Botanisches Institut, Naturwissenschaftl.  
Fakultät der Kaiserl. Universität Tokyo

### Literatur

- Kawada, Y. 1932. The double coiled spiral structure of chromosomes. Bot. Mag. (Tokyo) 46: 307-310 (Japanisch).  
Kuwada, Y. and T. Nakamura 1934a. Behaviour of chromonemata in mitosis. II. Artificial unravelling of coiled chromonemata. Cytologia 5: 244-247.  
— 1934b. Dieselbe III. Observation of living staminate hair cells in *Tradescantia reflexa*. Memo. Coll. Sci. Kyoto Imp. Univ. Ser. B. 9: 343-366.  
Rosenfeld, M. 1933. Experimental modification of mitosis by ammonia. Arch. exper. Zellforschg 14: 1-13.



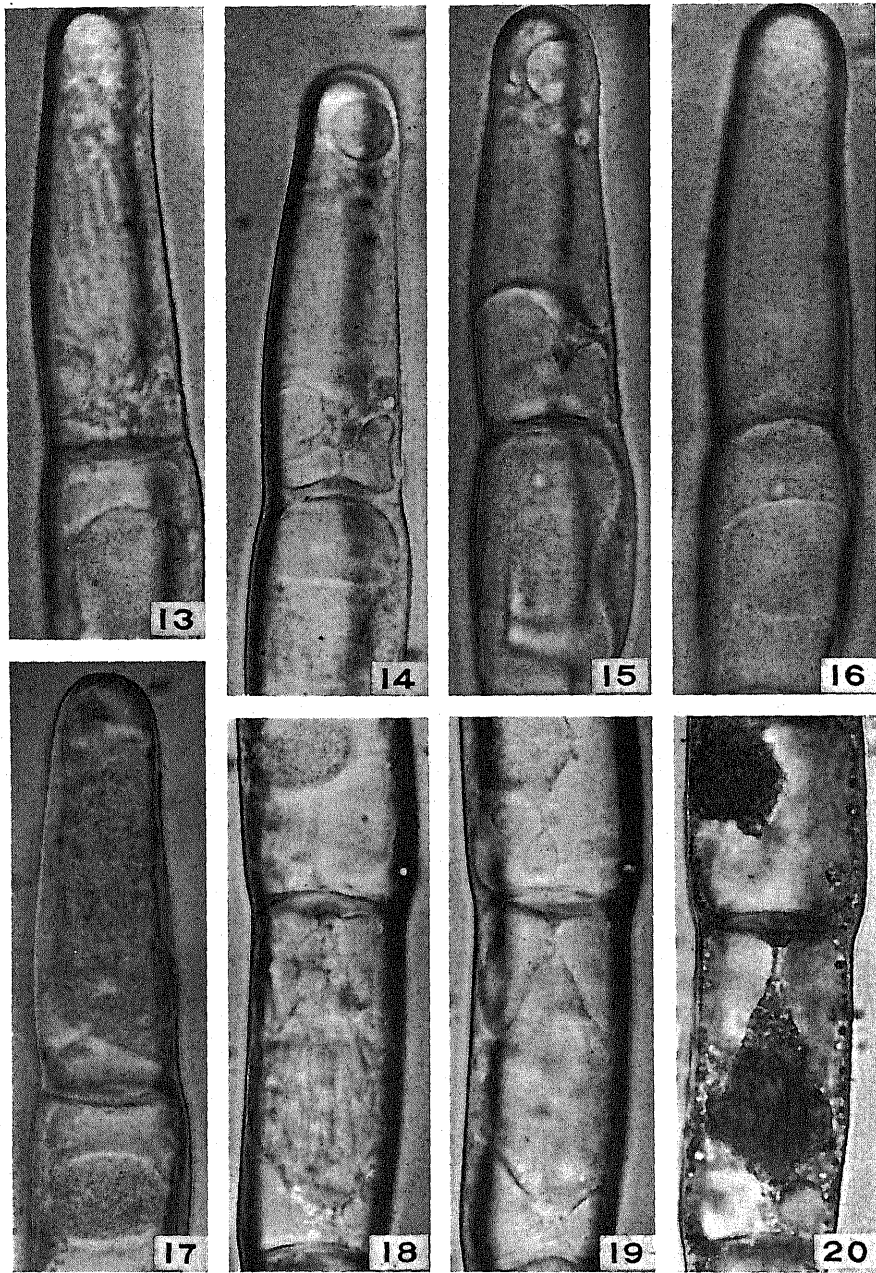


Wada: Mikrurgische Untersuchungen lebender Zellen in der Teilung. V.  
Die Einwirkung des Ammonia-Dampfes auf die Mitose usw.





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Wada: Mikrurgische Untersuchungen lebender Zellen in der Teilung. V.  
Die Einwirkung des Ammonia-Dampfes auf die Mitose usw.

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- Wada, B. 1932. Mikrurgische Untersuchungen lebender Zellen in der Teilung. I. Die Bildung von Tetraploidkernen, zweikernigen Zellen und anderen abnormen Teilungsbildern. *Cytologia* 4: 114-134.
- 1935. Dieselbe II. Das Verhalten der Spindelfigur und einige ihrer physikalischen Eigenschaften in den somatischen Zellen. *Cytologia* 6: 381-406.
- 1936a. Dieselbe III. Die Einwirkung der Plasmolyse auf die Mitose bei den Staubfadenhaarzellen von *Tradescantia reflexa*. *Cytologia* 7: 198-212.
- 1936b. Dieselbe IV. Die Einwirkung der Austrocknung auf die Mitose bei den Staubfadenhaarzellen von *Tradescantia reflexa*. *Cytologia* 7: 363-370.

#### Tafelerklärung

Alle Mikrophotographien wurden mit Hilfe von LEITZ „Makam“ unter Benutzung von LEITZ 1/12 Öl-Immersionsobjektiv und einem periplanen Okular 10× aufgenommen und in der Größe des originalen Negativs reproduziert. Ausführliche Erklärung der Figuren im Text. Vergrößerung ca. 940×.

#### Tafel 27

- Fig. 1-4. Wirkung des Ammonia-Dampfes auf Chromatinfäden. 1. Vor der Behandlung. Frühe Prophase. 2. Angequollener Zustand der Chromatinfäden und des Zytoplasmas. 3. Metakinese. Die Bildung der Chromosomen vollendet. 4. Ausgebildete Tochterzellen.
- Fig. 5-8. Wirkung des Ammonia-Dampfes auf Chromosomen. 5. Vor der Behandlung. Metakinese. 6. Angequollener Zustand der Chromosomen und des Zytoplasmas. In den Vakuolen tritt eine Anzahl von sich bewegenden Fällungen auf. 7. Späte Anaphase. Die Zytoplasmagliederung hat sich noch nicht vollständig erholt. Ein Spindelpol, der untere in der Figur, dringt daher in eine von den vergrößerten Vakuolen ein. 8. Gegen zwei und eine halbe Stunde nach der Behandlung ist die Kern- und Zellteilung vollendet.
- Fig. 9-12. Chromosomenaggregat. 9. Vor der Behandlung. Mittlere Anaphase. 10. Angequollener Zustand der Chromosomen und des Zytoplasmas. 11. Die Chromosomen treffen zu einem Klumpen zusammen. 12. Ein Tag nach der Behandlung. In der Zelle entsteht ein Aggregat von Chromosomen.

#### Tafel 28

- Fig. 13-17. Zweimalige Quellung einer Teilungsfigur. 13. Vor der Behandlung. Mittlere Anaphase. 14. Die erste Quellung der Chromosomen und Zytoplasmas. 15. Fünf Minuten nach der Behandlung. Entmischung der Chromosomen und des Zytoplasmas. 16. Die zweite Quellung der Teilungsfigur und des Zytoplasmas. 17. Azetokarminfärbung. Die durch die zweite Quellung aufgefaserte Struktur der Chromosomen ähnelt der Struktur eines Ruhekernes.
- Fig. 18-20. Azetokarminfärbung angequollener Chromosomen. 18. Vor der Behandlung. Mittlere Anaphase. 19. Angequollener Zustand der Chromosomen, wobei die Spindel noch ihre Gestalt beibehält. 20. Azetokarminfärbung. Die Spiralstruktur der Chromosomen ist noch erkennbar.

**Absence of the Y-Chromosome in the Vole, *Microtus montebelli* Edw.  
with Supplementary Remarks on the Sex-Chromosomes  
of *Evotomys* and *Apodemus*<sup>1)</sup>**

By

**Kan Oguma**

Zool. Inst., Hokkaido Imp. Univ., Sapporo

( With 3 Text figures and one Plate )

Shortly after publication of the present writer's work (1935) on the sex-chromosomes of a vole, *Evotomys bedfordiae*, in which the absence of the Y-chromosome was observed, Matthey and Renaud (1935) produced a short account of the sex-chromosomes of *Evotomys glareolus*, announcing, in contrast to the present author's results, the existence of the Y-chromosome as a mate of the X, and emphasizing, at the same time, the idea of the constant occurrence of the XY-type of sex-chromosomes through all kinds of Mammalia. Such an idea has already been elaborated by many cytologists since Painter (1925) claimed it for the first time.

Adverse to such an opinion, the writer holds that the sex-chromosomes of Mammalia may sometimes be XY-type, or sometimes XO-type, as in the course of more widely extended observations examples were found in which the Y-chromosome is really absent. Especially among the rodents, *Apodemus speciosus ainu* (1934) and *Evotomys bedfordiae* (1935) exhibit XO-type of the sex-chromosomes as the writer believes in striking contrast to species belonging to the genera *Rattus* and *Mus*, in which also the XY-complex of the sex-chromosomes was found by the present writer.

It is not surprising to the writer to know that his statements obtained from *Apodemus* and *Evotomys* may sometimes be considered by some authors as if resulting from an erroneous interpretation, because, they certainly disturb to a great extent the idea of the constant occurrence of the Y-chromosome throughout a definite systematic group of animals. But the writer still feels, after repeated observation of his slides, that no revision or alteration of his ideas is necessitated. Furthermore, the writer's chromosome survey of Japanese wild Muridae has been extended to discover one more example in which the Y-chromosome is certainly absent. That is a

1) Contribution No. 111 from the Zoological Institute, Faculty of Science, Hokkaido Imperial University.

vole possessing the scientific name, *Microtus montebelli* Edw.,<sup>1)</sup> belonging to the subfamily Microtinae together with *Evotomys* and *Arvicola*. The vole lives in farms in the main island of Japan and is well known not only from its injurious effect on crops but also as the propagator of certain local epidemics. It is the chief purpose of the present paper to communicate what could be observed in the chromosomes of this species of Muridae, but some additional comments will be made upon previous results obtained from the studies on *Evotomys* and *Apodemus*, as they have connection with the present findings.

### Morphological analysis of spermatogonial chromosomes

It will be sufficient to show here four metaphase plates of spermatogonia of the best fixation, selected from four different individuals, in order to determine the chromosome number  $2n$ , characteristic to the vole now dealt with (Text fig. 1, A-D; Pl. 29, Figs. 1-3). Without the slightest ambiguity, thirty-one chromosomes (an odd number) can be counted in every single garniture shown in the figures. The same number is further proved in many other spermatogonia which are not here reproduced, and it will be noticed that this is one of the smallest number so far recorded from Mammalia (*vide infra*). In addition to such a small number, the chromosomes, as well as the cell in which they are included, are remarkably large in size. This, therefore, makes the counting very easy and allows further morphological analysis, by which the homologous mates are discriminated. The chromosomes assume V-shape in majority, in striking contrast to all other species of Muridae hitherto studied, except one case, *Arvicola scherman*,<sup>2)</sup> in which similarly shaped chromosomes have recently been informed by Matthey and Renaud (1935). In the figures shown in Text fig. 1, the present writer has tried to label with a's to o's the thirty autosomes constituting fifteen pairs, after close comparison of their size and shape, and one X for the single sex-chromosome.

1) Material was collected and prepared at Niigata through the cordial assistance of Dr. K. Ikeda, to whom sincerest thanks are now expressed. Fourteen testes obtained from seven adult males were fixed by (a) Flemming without acetic acid and (b) Champy, but the best figures of chromosomes were confined in material treated with the former fluid. Sections were stained by iron haematoxylin after Heidenhain with counter staining of light green.

2) In their original paper Matthey and Renaud (1935) adopted the name *Arvicola scherman terrestris* L. But the species employed by them as the material of their study seems to have correctly the name *Arvicola scherman exitus* Miller, indigenous to Switzerland (cf. Miller's Catalogue of the Mammals of western Europe, pp. 746-749, 1912, and Hinton's Monograph of Voles, pp. 410-414, 1926).

*Chromosome pair a.* They are sharply characterized by their exceedingly huge size as compared with the remainder, presenting a V-shape with two arms of considerably different length, the fibre attachment being submedian. The longer arm usually extends twice as long as the shorter (In Text fig. 1, A, one of the a's, found in upper left hand quarter, appears to be mediomitic by the apparent shortening of the longer arm, of which the terminal part is bent in the direction of vision). In equatorial arrangement they are found in major cases at opposite positions in the peripheral zone though approaching in some instance as Text fig. 1, C.

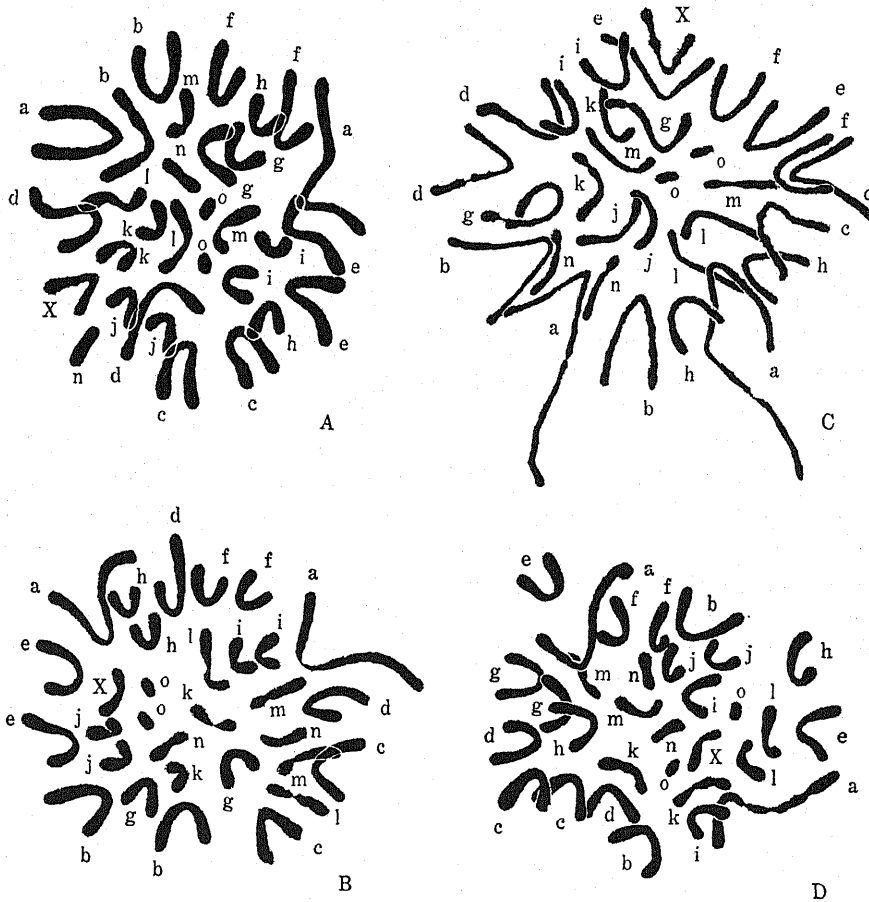
*Chromosome pairs b to k.* These twenty chromosome constituting ten homologous pairs are also represented by V's but considerably smaller than the a's. They show gradatory diminution of length from b to k in alphabetical order, with the apicis of the V's directed towards the center of the equator. Every two chromosomes regarded as homologous usually lie near each other with occasional exceptions. Among these ten pairs some possess a median fibre attachment or mediomitic, having two arms of equal length, while some are of submedian attachment, having consequently two arms of different length. It is not absolutely certain at present how many pairs out of the ten belong to the former kind, but two pairs b and c, at least, seem invariably to have arms of equal length. In some of the latter kind a terminal constriction in the longer of one V can frequently be observed. A terminal portion, thus constricted off from the main bulk of the arm, becomes occasionally separated from the latter having an achromatic connection. Chromosome pairs d and g (Text fig. 1, C) for instance show very clearly such occurrence, and as the satellite structure in chromosomes of some rodents as squirrels as described by Cross (1931) is recalled.

*Chromosome pair l.* These chromosomes may sometimes be longer than the k, but they have a subterminal fibre attachment, instead of submedian as those of the preceding category, and thereby assume a J-shape with terminal bendings.

*Chromosome pair m.* They seem like telomitic rod-chromosomes with a constriction near one extremity in each. At the point of constriction they bend to give rise to the J-shape as chromosomes l, in approximately the same frequency with the case where they do not show bending (Compare m's in Text fig. 1, A-D). It is not certain at present whether they should be classed as telomitic chromosomes or atelomitic ones in which the spindle fibres should be found at the point of constriction.



*Chromosome pair n.* They show also an ambiguous figure in respect to fibre attachment as do chromosomes *m*. With most probability, however, they are telomitic rod-chromosomes having satellite structure at one extremity, although this structure becomes obscure when the chromosomes acquire heavy condensation (Text fig. 1, A).



Text fig. 1. *Microtus montebelli*. Chromosomes in metaphase of spermatogonial division (polar view).  $\times$ ca 5500.

*Chromosome pair o.* The smallest chromosomes thus designated by the letter "o", are obviously rod-chromosomes with a terminal attachment of spindle fibre. They attain a length not more than one third that of chromosomes *n*, and by this minor size they show so striking feature in a spermatogonial garniture as the *m*-chromosomes, which are not noticeable in any species of Muridae. Corresponding to the *m*-chromosomes in other kinds of animals these



"o" chromosomes of minute size always arrange themselves at the central region of the equatorial plate.

To summarize the above descriptions the whole thirty chromosomes in fifteen pairs are classified into three different kinds in their apparent configuration as follows:

V-chromosomes (V's), a, b, c, d, e, f, g, h, i, j, k.

J-chromosomes (J's), l, m.

Rod-chromosomes (R's), n, o.

In the V's the spindle fibre attaches beyond doubt at the bent point or on the apex of the V, while in the R's it is inserted at the terminal part as indicated telomitic. The J's, on the contrary, may or may not have the fibre attachment at the point of bending. In the l chromosomes for instance the fibre attachment seems with all probability to be subterminal, but in the m's the matter is different, for, in a large number of cases the entire body of the chromosome is stretched into a straight rod, showing no bending, and the terminal globular parts are invariably directed to the center of the equatorial plate as ordinary R's do, but in minor cases they clearly show bending and thereby present the J-shape. If the essential structure of the m's be telomitic, then the J-shape thus produced can not be accounted for otherwise than as a temporal and superficial figure, and it should be distinguished from the true J's of atelomic nature.

*Sex-chromosome.* After labelling a's to o's for the fifteen pairs of autosomes as mentioned above, there is eventually left a single chromosome destitute of its synaptic mate. This corresponds to the sex-chromosome as labelled "X" in the figures. The X-chromosome thus identified is found in major cases (though not absolutely) on the peripheral zone of equatorial arrangement of chromosomes. In respect to size it belongs to the chromosome of medium size with approximately similar magnitude to the J-chromosomes, and assumes, without exception, a V-shape, of which one arm is remarkably longer than the other arm. It has a characteristic of remaining somewhat vague in contour or not heavily condensed as compared with the autosomes. Moreover, special attention should be called to the fact that in the longer arm of this V-shaped X-chromosome are frequently visible two partial swellings of chromatin (Text fig. 1, C), which are almost universally observable in X-chromosomes of Muridae as well as in that of man, and will be referred to again later.

There is no other sex-chromosome corresponding to the Y-chromosome. The figures shown in Text fig. 1 are so clear as to show undoubtedly the absence of the Y, and in reality the diploid group of chromosomes does not involve a sex-chromosome in addition to the X, showing an odd number 31.

### Tetrads and the X-chromosome in the primary spermatocytes

When observed from the pole of division the primary spermatocyte always shows sixteen chromosomes, of which fifteen are bivalent autosomes or tetrads and the remaining one is the univalent X-chromosome (Text fig. 2, A and B). The former present very characteristic and different forms from those of ordinary *Rattus* or *Mus*. A majority appear like thick crosses, or, to put it otherwise, one bivalent is composed of two thick V's connected with each other of their apices. A few, however, still take the form of horizontal rings which usually occur in descendants of simple rod-chromosomes found in other species of Muridae. The smallest chromosomes, the o's, are of course noticeable as the smallest bivalent with the appearance of a solid dumbbell.

In striking contrast to those bivalents described above, the X-chromosome remains in univalent condition destitute of its synaptic mate, and in good preparations it is sharply distinguished from the autosome bivalents not only by its V-shape but also by its less affinity to haematoxylin. In well differentiated preparations it takes a gray colour while the autosomes are stained deep black (Text fig. 2, A). Such a peculiarity in respect to stains has been known in many different kinds of animals covering both vertebrata and invertebrata. Before trying further descriptions of its morphology the autosomes will be described from the side of equatorial arrangement. In profile the essential structure of the bivalents will first clearly be understood, especially in rather strongly destained preparations (Text-fig. 2, C; Pl. 29, Fig. 4). In short, most of them belong to vertical ring-tetrads, similar to those of urodelan Amphibia, but there are produced many variations caused chiefly by the occurrence of chiasmata.

Now to continue about the morphology of the X-chromosome in metaphase. As already known from the polar view of the metaphase plate, the X-chromosome takes a V form, and the two component arms differ from each other in thickness as well as in length. The thicker arm is always longer than the thinner one, the former assuming a clavate shape while the latter usually keeps the same diameter throughout its entire length (Text fig. 2, A, D and F; Pl. 29, Figs. 5 and 7). The former corresponds to the distal segment and the latter to the proximal segment in terminology of the segmentary structure of X-chromosomes as is discussed in another paper (in press). The present writer has described in that paper, how in the X-chromosome of man and rodents the distal segment is frequently subdivided

into two distinct parts,  $D_1$  and  $D_2$ , while the proximal segment always remains in single P, which may sometimes grow thinner having a small spherical swelling at the extremity, or sometimes become con-



Text fig. 2. *Microtus montebelli*. Chromosomes in metaphase and anaphase of primary spermatocytes in division.  $\times$  ca 5500. A and B, metaphase, polar view. C, D, E, F, metaphase, lateral view. G and H, anaphase, lateral view, notice the lagging X-chromosome of pale colour.

verted into a comparatively large globule at the expense of the elongate part. It is in the latter case that the P-segment is very frequently regarded as if it were the Y-chromosome. A feature, suiting the above descriptions, can also be discovered in the case of the present vole. The X-chromosome shown in Text fig. 1, E (= Pl. 29, Fig. 6) for instance, strongly resembles the XY-complex, but in

reality the two contiguous segments (upper part) represent the  $D_1$  and the  $D_2$  parts, while the single segment (lower part) having chromatic connection with the former is nothing more than the P-segment, not to be considered as the Y. It is of great importance to know that such a tripartite structure,  $D_1$ - $D_2$ -P, can be followed back to the spermatogonial stage (Text fig. 1, C). In case where the chromosomes are too much destained and therefore become thinner in general, one will not overlook the presence of the proximal granule at the junction of  $D_1$  and P (Text fig. 2, C and H; Pl. 29, Fig. 4).

In parallel to the tripartite structure displayed by the X-chromosome in question, it is very important to observe in detail its behavior during meiosis. Up to the commencement of the separation of dyads, the X-chromosome occupies its position on the same plane with tetrads, hesitating to move to the pole with the separated dyads (Text fig. 2, G). When the latter approach, however, to the poles of division to which they belong, the X-chromosome starts to follow after one of the daughter groups of chromosomes (Text fig. 2, H). No disintegration occurs between the D- and P-segment of the X-chromosome, as both two component segments, with a proximal granule between them, are clearly observable in the same configuration and constitution as in the previous stage (Text fig. 2, G). By this phenomenon it will be made absolutely clear that the P-segment is by no means to be considered the Y-chromosome, but merely a definite part of the X-chromosome, discriminated as the P-segment by the present writer.

### Discussion

*On the chromosome number.* To the author of this paper it came as a surprise when Krallinger (1936) informed the chromosome number of *Pecari tajacu*, in which he found only 30 chromosomes in diploid, a small number incomparable with any species of Eutheria hitherto recorded. But soon Koller (1936) discovered the still lower chromosome number, 28, in the grey squirrel, *Sciurus carolinensis leuconotus*. Thus our knowledge on the chromosome number has gradually been accumulated and the previous idea concerning the fundamental number of Eutheria chromosomes had necessarily been altered and revised. At present 28 found in the gray squirrel holds, at any rate, as the lowest chromosome number in Eutheria, followed by 30 in *Pecari* and then the number 31 now found in the vole, *Microtus montebelli*.

Knowledge on chromosomes of any species belonging to *Microtus* has been very meager, as only a short account is available from one

of American species, *Microtus townsendii*, by Cross (1931) who reports the chromosome number ( $2n$ ) of this species as 50, of which all appear as rods and spherules. In the present species, *Microtus montebelli*, on the contrary, the chromosome number is remarkably lower being only 31, and a majority of the chromosomes are represented (at least eleven pairs from a to k) by V's which may probably be multiples. As these eleven pairs may be explained as multiples, however, the chromosome numbers of these two different species can closely approach each other, by analysing each V into two rods. Such an analysis of V-chromosomes seems to be more reasonable when an attempt is made to compare the present species with *Evotomys bedfordiae*, in which the writer has already recorded (1935) 55 chromosomes of rod-shape. For, if the twelve pairs of V and J chromosomes, a's to l's, be considered as multiples, then there is obtained 48 R's (rods) by breaking every individual V into two R's. This number 48 will suffice to give rise to the number 55, similar to that of *Evotomys bedfordiae*, by adding 6 remaining autosomes, (m's, n's and o's) and one X-chromosome. But there remains a great doubt whether the chromosomes m's are essentially rod-chromosomes or whether the l's are in reality multiples.

In respect to chromosome morphology, *Microtus montebelli* is much more closely allied to *Arvicola scherman* studied recently by Matthey and Renaud (1935) than to the American *Microtus* studied by Cross (1931) and *Evotomys*. Because, they found 36 chromosomes in *Arvicola scherman*, a majority of which being V-shape. Their information, to the regret of the present writer, is confined to a very short note, not extending so far as to allow any morphological analysis and therefore it is impossible to know at present how many V's are actually present in a garniture of chromosomes.

*On the problem of the sex-chromosomes.* As stated in the introduction to this memoir, the present writer holds the view, adverse to most investigators of Mammalian chromosomes, that the formula of the sex-chromosomes varies in Mammalia either XY or XO in different species, and it seems very questionable whether the chromosome which is interpreted as XY-complex by some authors is always in reality of a multiple nature composed of two distinct chromosomes X and Y, or whether it merely possesses an appearance of a multiple chromosome as a result of segmentary construction.

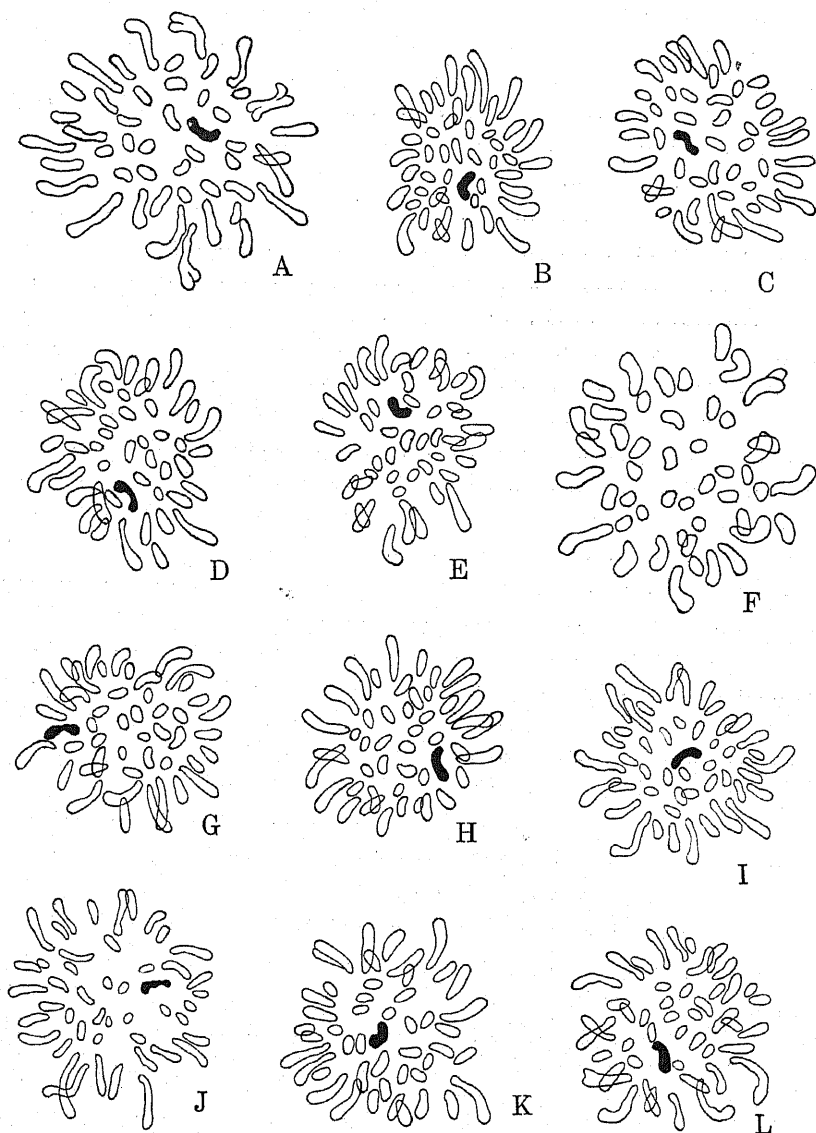
It is beyond doubt clear that in figures of spermatogonial chromosomes reproduced in the present paper no one can count 32 chromosomes instead of 31. What is meant by this fact of an odd number of chromosomes? Moreover, the X-chromosome, thus identified

by the writer, is constantly found in auxocytes with a constant configuration. In striking contrast to this, Matthey and Renaud (1935) found 36 chromosomes (diploid number) in *Arvicola scherman*, without any difficulties according to their statement, and one XY-complex, composed of two distinct chromosomes connected *bout à bout*, in the reduced garniture of chromosomes in the auxocyte. The writer does not know whether such a discrepancy is due to the taxonomical differences existing between an oriental and an occidental species of voles.

On the contrary, however, they doubt the writer's previous account of *Evotomys bedfordiae*, in which were described and figured 55 distinct chromosomes in the spermatogonium, as they presume the chromosome number of *Evotomys glareolus* to be probably 56 (they could not actually count it). They say that in auxocytes *il est aisé d'identifier un couple XY*. At present, the writer is quite ignorant of the morphology of that complex, as their memoir has no accompanying figures. If there exists homology between their species and the writer's, however, they might have considered the minute globular body attached to one end of X as a distinct Y-chromosome. That minute body with a globular appearance denotes, so far as observed in *Evotomys bedfordiae*, nothing other than the proximal granule to which the spindle fibre comes to attach itself. Because, if it be the Y-chromosome as they may interpret, eventually one extremely minute spherule in the spermatogonial garniture should be discovered, but unfortunately the writer can not find at all such a chromosome corresponding to that minute spherule (see previous paper, 1935, Figs. 25 and 26).

On closing of present discussion it seems necessary to the writer to touch on the problem of the sex-chromosomes of *Apodemus*, since he recorded (1934) for the first time that in *Apodemus speciosus ainu* the sex-chromosome separates postreductionally in a quite different manner from cases hitherto known in Mammalia. A similar behaviour has also been ascertained to occur in sex-chromosomes in several other species of *Apodemus*, by Tateishi (1934), Raynaud (1936) and Matthey (1936a, 1936b). But all these authors hold the view, different from the present writer's, that the short arm of the V-shaped sex-chromosome represents the Y-element of an XY-complex, instead of being a part of a single X-chromosome, as the writer holds. The writer's opinion is based upon two valid facts: (1) both arms, long and short, of a V-shaped chromosome develop within one and the same chromosome vesicle, and (2) the diploid number of chromosomes found in the spermatogonium is 47, among which is invariably observed one V-shaped chromosome with a correspondent

configuration to some extent with the sex-chromosome in auxocytes. In the writer's previous paper (1934) the spermatogonial garniture of chromosomes was shown by only one example. It is with reference



Text fig. 3. *Apodemus speciosus ainu*. Chromosomes in metaphase of spermatogonial division (polar view).  $\times$  ca 3000. X-chromosomes are indicated by solid black; it is not distinguishable in F, for all chromosomes are extremely thickened.

to this case that Matthey (1936a, 1936b) expressed unwillingness to accept the writer's interpretation, including doubt that there may exist cells in which 48 chromosomes compose a diploid garniture, as



Raynaud (1936) actually sketches from *Apodemus sylvaticus* (only one figure is shown by the latter author and no V-shaped chromosome is visible). The single figure shown in the writer's previous paper does not represent the sole example from which the conclusion was drawn, but is only one representative out of a great many cells of best fixation under close observation. On the present occasion, therefore, the writer wishes to publish here, with a supplementary purpose, some of his sketches of spermatogonia (Text fig. 3; Pl. 29, Figs. 8-10) from the same material, in which every single garniture is invariably composed of 47 distinct chromosomes and in most of them is noticed a chromosome having a more or less V-shape (solid black in figures) and without any apparent mate.

Thus the writer's comparative study of rodents results in a conclusion that the Y-chromosome is really absent in one field mouse, *Apodemus speciosus ainu*, and two voles, *Evotomys bedfordiae* and *Microtus montebelli*. This is striking contrast to the house mouse, *Mus molossinus*, and the rats, *Rattus norvegicus* and *Rattus rattus*, in all which species the Y-chromosome is proved to exist. It is advisable to any one who investigates the Mammalian sex-chromosomes, before he decides to explain a small chromatic segment as the Y-chromosome, that he should pay special attention to the segmentary structure of the X-chromosome as discussed in detail in another paper by the present author (in press) in connection with the human sex-chromosome. The X-chromosome seems in general to show a tripartite structure not only in man and in rats, but also in species of *Lepus* as have recently been studied by Tateishi (1936). Occasion will be found in the near future to discuss again the problem of the sex-chromosome, because a study of the chromosomes is going on in another species of *Apodemus*, indigenous to Japan.

#### Literature cited

- Cross, J. C. 1931. A comparative study of the chromosomes of rodents. Journ. Morph., Vol. 52.
- Koller, P. C. 1936. Cytological studies on the reproductive organs. Chromosome behaviour in the male grey squirrel (*Sciurus carolinensis leucotus*). Proc. Roy. Soc. Edinburch., Vol. 56, Pt. II.
- Krallinger, H. F. 1936. Die Chromosomen des Halsbandpekaris (*Pecari tajacu*). Zeits. Zellf. mikr. Anat., Bd. 24.
- Matthey, R. 1936a. Le problem des hétérochromosomes chez les Mammifères. Arch. Biol., T. 47.
- 1936b. La formule chromosomiale et les hétérochromosomes chez les *Apodemus* Européens. Zeits. Zellf. mikr. Anat., Bd. 25.
- Matthey, R. and P. Renaud 1935. Le type de digamétie male et les chromosomes chez deux campagnols. Comp. Rend. Séan. Soc. Biol., T. 120, p. 595.
- Oguma, K. 1934. A new type of the mammalian sex-chromosome, found in a field mouse, *Apodemus speciosus*. Cytologia, Vol. 5.



- Oguma, K. 1935. The chromosomes of four wild species of Muridae. Jour. Fac. Sc. Hokkaido Imp. Univ., Ser. VI, Vol. 4.
- Painter, T. S. 1925. A comparative study of the chromosomes of Mammals. Amer. Nat., Vol. 59.
- Raynaud, M. A. 1936. Les hétérochromosomes du mulot. Comp. Rend. Séan. Acad. Sc., T. 202, p. 1942.
- Tateishi, S. 1934. A preliminary report on some peculiarly shaped chromosomes in three species of *Apodemus*. Trans. Nat. Hist. Formosa, 24.
- 1936. The chromosomes of Formosan hare, *Lepus formosus*, and of the rabbit, *Lepus cuniculus*. Zool. Mag. (Dobutsugaku Zasshi), Vol. 48.

#### Explanation of Plate 29

All photomicrographs were taken under magnification  $\times 2000$ .

##### *Microtus montebelli*

Figs. 1-3. Spermatogonial chromosomes (polar view).

1. corresponds with Text fig. 1, A
2. " " " " B
3. " " " " C

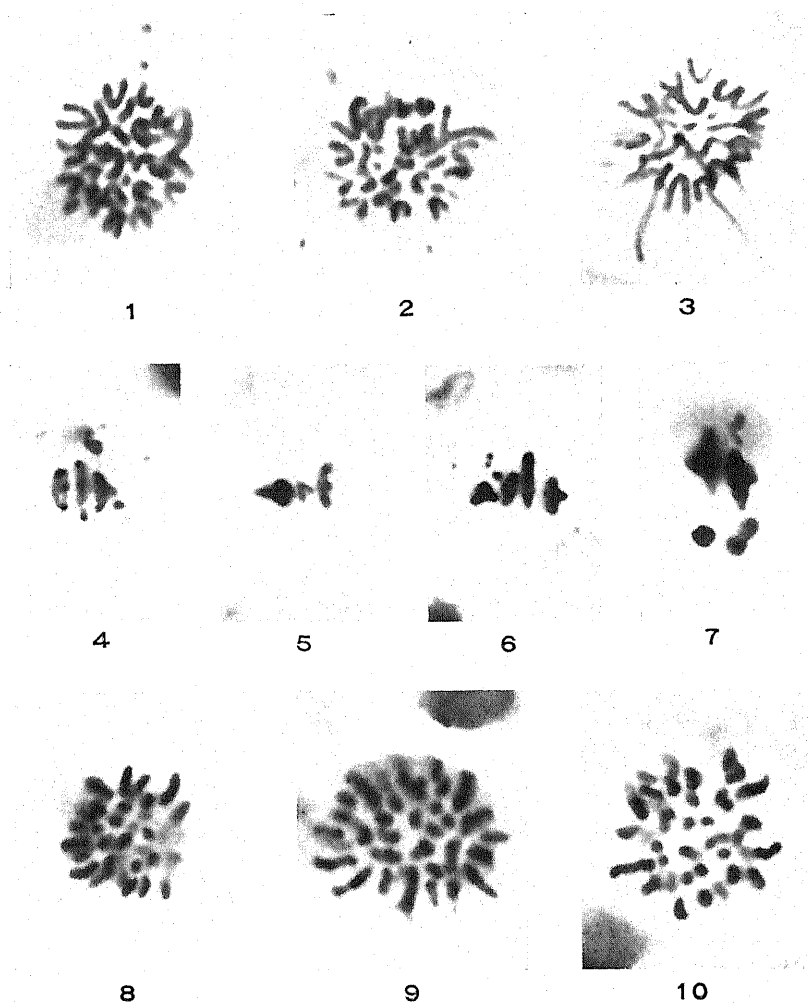
Figs. 4-7. Chromosomes of primary spermatocytes (profile),  $x$  denotes the X-chromosome.

4. corresponds with Text fig. 2, C
5. " " " " D
6. " " " " E
7. " " " " F

##### *Apodemus speciosus ainu*

Figs. 8-10. Spermatogonial chromosomes (polar view).

8. corresponds with Text fig. 3, K
9. " " " " A
10. " " " " F



Oguma: Absence of the Y-Chromosome in the Vole, *Microtus montebelli*  
Edw. with Supplementary Remarks on the Sex-Chromosomes of  
*Evotomys* and *Apodemus*



## Sur la coloration vitale des vacuoles par le rouge neutre dans les cellules du protonéma de "*Polytrichum commune*"

par

A. Guilliermond

Rappelons qu'à la suite d'importantes recherches faites à l'aide des colorations vitales au rouge neutre, Parat et ses collaborateurs, puis ensuite beaucoup d'autres auteurs ont mis en évidence la présence de vacuoles dans les cellules animales les plus diverses, où jusqu'alors on ignorait la présence de ces éléments. Ces vacuoles sont généralement à l'état de petites inclusions disséminées dans le cytoplasme et formées par une solution colloïdale très concentrée, c'est-à-dire assez semblables à celles que l'on observe dans les cellules embryonnaires des végétaux; dans quelques cas cependant, elles peuvent être volumineuses, à contenu liquide et présenter le même aspect que dans les cellules végétales adultes.

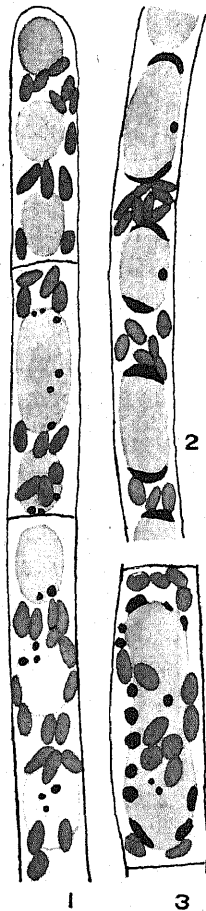
Ces données ont été l'objet de contestations, notamment de la part de Beams qui admet que les cellules animales ne renferment normalement aucune vacuole et que les inclusions qui ont été décrites au moyen de colorations vitales par le rouge neutre sont des vacuoles artificielles créées par le colorant.

Eliot Weier a essayé récemment de transporter la théorie de Beams dans le domaine de la cytologie végétale. Cet auteur a étudié l'action du rouge neutre sur les cellules du protonéma de *Polytrichum commune*. Or, il a constaté que ce colorant amène d'abord la formation de granulations fortement colorées dans le cytoplasme autour de la vacuole et non dans son intérieur. Ces granulations, qu'il assimile aux vacuoles artificielles de Beams et aux vacuoles de Parat, grossissent peu à peu, puis émigrent ensuite dans la vacuole où elles se dissolvent en provoquant la coloration diffuse du suc vacuolaire. Bien que la coloration vitale des vacuoles dans les cellules végétales soit aujourd'hui un fait démontré qu'il n'est pas possible de contester,<sup>1)</sup>

1) Nos recherches faites sur les végétaux les plus divers ont prouvé que le rouge neutre ne provoque jamais aucune production artificielle dans le cytoplasme qu'il traverse sans le colorer pour s'accumuler exclusivement dans la vacuole. Elles ont démontré également que les colorants vitaux, en particulier le rouge neutre sont aussi peu toxiques que possible. Nous avons pu, en effet, réaliser la culture d'un *Saprolegnia* en milieu nutritif additionné de rouge neutre et observer l'évolution des vacuoles colorées pendant tout le développement du Champignon, depuis la germination des zoospores jusqu'à la formation des zoosporanges. Enfin, nous avons également obtenu la germination des graines de Tabac (Guilliermond, Dufrénoy et Labrousse) et des graines d'Orge dans des solutions de rouge neutre (Guilliermond et Obaton).

nous avons tenu à reprendre les observations d'Eliot Weier sur les cellules du protonéma de *Polytrichum commune*.

En colorant vitalement par le rouge neutre les cellules de ce protonéma et en suivant sous le microscope la marche de la coloration, nous avons pu constater la formation immédiate au sein de la vacuole et non du cytoplasme, de nombreuses granulations fortement colorées et animées de mouvements browniens; celles-ci, d'abord très petites, grossissent peu à peu en se fusionnant les unes dans les autres,



puis viennent s'accoler sur le bord de la vacuole; dans quelques cas même, elles peuvent émigrer dans le cytoplasme périvacuolaire (Fig. 1). Si le colorant se trouve à un certain degré de concentration, ces granulations finissent par se dissoudre dans le suc vacuolaire et déterminent la coloration diffuse de la vacuole. Tels sont les phénomènes que nous avons pu suivre sous le microscope et qui montrent l'inexactitude des observations d'Eliot Weier. Nos observations prouvent donc, de la manière la plus nette, que les granulations formées sous l'action du rouge neutre ne sont pas localisées dans le cytoplasme, mais dans la vacuole elle-même et résultent de la floculation d'une substance colloïdale se trouvant en pseudo-solution dans le suc vacuolaire.

Fig. 1. Fragments de filaments de protonéma de *Polytrichum commune* colorés vitalement par le rouge neutre—1: dans la cellule inférieure les vacuoles ont formé dans leur intérieur des granulations fortement colorées et animées de mouvements browniens—les autres vacuoles du filament renferment des granulations fortement colorées et ont en même temps leur suc teint de manière diffuse; dans la cellule supérieure, il n'y a pas de granulations, mais seulement une coloration diffuse du suc vacuolaire; 2—les granulations formées sous l'action du colorant se sont fusionnées et forment des calottes aux pôles des vacuoles dont le suc est déjà coloré d'une manière diffuse. 3—Les précipités vacuolaires tendent à émigrer dans le cytoplasme périvacuolaire.

Les phénomènes se passent dans les vacuoles du protonéma de *Polytrichum commune* exactement comme dans les Levures et dans la plupart des Champignons. Dans des recherches, qu'Eliot Weier semble ignorer, nous avons décrit, en effet, avec beaucoup de précision, dans divers Champignons (*Penicillium glaucum*, *Oidium lactis* et surtout dans le *Saccharomyces Ludwigii*), en les suivant sous le microscope, les diverses phases de la coloration vitale. Celle-ci

commence d'abord par la production, dans la vacuole, de nombreuses et très fines granulations fortement colorées et animées de mouvements browniens (Fig. 2); ces granulations se fusionnent ensuite

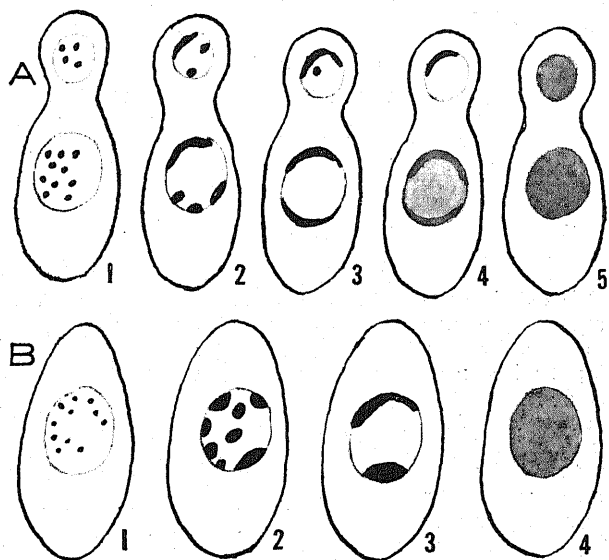


Fig. 2. Cellules de *Saccharomyces Ludwigii* observées sous le microscope pendant la coloration vitale par le rouge neutre. A—1; début: formation dans la vacuole de nombreux précipités fortement colorés. 2 et 3: les précipités se fusionnent et s'accumulent contre la paroi de la membrane vacuolaire. 4 et 5: dissolution des précipités et coloration diffuse du suc vacuolaire. B: Début: précipitation du colloïde. 2 et 3: fusionnement des grains. 4: dissolution des précipités.

les unes dans les autres pour former un petit nombre de gros globules (souvent un seul), qui viennent s'accoler sur le bord de la vacuole dont ils épousent le contour, prenant la forme de croissant, puis finissent par se dissoudre en communiquant à la vacuole toute entière une teinte rouge diffuse qui s'accroît progressivement par accumulation du rouge neutre.

Nous avons montré toutefois que pour que toutes ces phases successives de la coloration s'accomplissent, il est nécessaire que le rouge neutre se trouve à une concentration suffisante dans la solution: si la coloration est faite avec une solution très diluée du colorant, il y a simplement formation, dans la vacuole, de nombreuses granulations animées de mouvements browniens et souvent, par suite d'un phénomène physique qu'il resterait à expliquer, une partie de ces granulations peut émigrer dans le cytoplasme périvacuolaire (Fig. 3). A une concentration plus forte de rouge neutre, on constate simplement la confluence de ces granulations en un petit nombre de globules plus gros et, enfin, une concentration un peu plus élevée encore pro-

voque la dissolution de ces globules et la coloration totale de la vacuole. Nous avons comparé ces phénomènes au schéma décrit par von

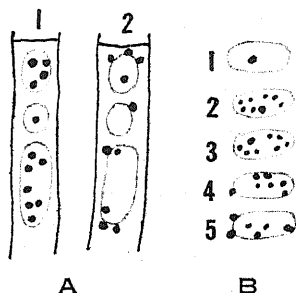


Fig. 3. A : 1—début de la coloration vitale par le rouge neutre dans un fragment de filament d'*Oidium lactis*; 2: les précipités émigrent de la vacuole dans le cytoplasme. B: Début de la coloration vitale dans une vacuole: formation de précipités dans la même vacuole et émigration de ceux-ci dans le cytoplasme. Ce phénomène s'observe surtout quand le colorant est à l'état de solution très diluée.

Möllendorff de la coloration vitale des cellules animales par le rouge neutre. Ce savant a montré que dans ces cellules le rouge neutre s'accumule dans de petites inclusions colloïdales fluides du cytoplasme (vacuoles de Parat) dont il provoque la floculation de la substance colloïdale sous forme de précipités fortement colorés, suivie ensuite de la redissolution des précipités et de la coloration totale de l'inclusion. Von Möllendorff explique les phénomènes en admettant que le colorant basique provoque la floculation du colloïde acide de l'inclusion cytoplasmique, puis que, si le colorant se trouve en excès sur le colloïde de l'inclusion, il lui communique sa charge d'où redissolution du précipité (Fig. 4).

Il semble que le raisonnement de von Möllendorff peut être appliqué à la coloration vitale des vacuoles des Cham-

pignons et des cellules de *Polytrichum commune*.

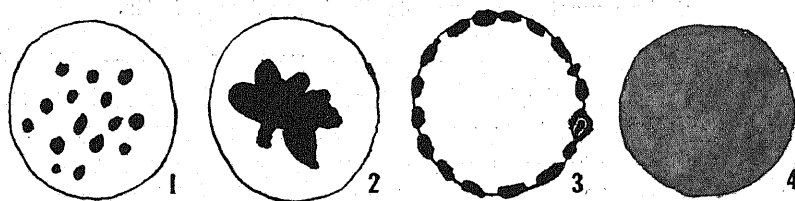


Fig. 4. Schéma de la coloration vitale dans les cellules animales par von Möllendorff : 1, 2 et 3: inclusions fluides du cytoplasme: début de la coloration et formation de précipités colorés dans l'inclusion. 4: dissolution des précipités.

Cependant, le schéma de von Möllendorff ne saurait être généralisé, car il ne s'applique pas à la coloration vitale des vacuoles de certains Végétaux. Il y a, en effet, des cellules, comme celles notamment des racines d'Orge et de Blé, les épidermes de divers organes de Lis etc., dont les vacuoles semblent renfermer surtout une pseudo-solution de phospholipides ou un complexe phospholipides-stériles, dans lesquelles le rouge neutre ne provoque ordinairement la formation d'aucun précipité: dans ces cellules, la coloration diffuse de la vacuole s'établit d'emblée. Les colorants vitaux étant plus solubles



dans les phospholipides et les stérides, on pourrait envisager un coefficient de partage de ceux-ci (Reilhaes). Il semble donc que la coloration vitale des vacuoles ne s'effectue pas toujours par le même mécanisme et dépend de la nature chimique des substances colloïdales qui se trouvent en pseudo-solution dans le suc vacuolaire, substances qui, ainsi que nous l'avons démontré, diffèrent du tout au tout d'un type de cellules à l'autre.

Quoiqu'il en soit, on voit donc que les observations d'Eliot Weier sont inexactes et nous pensons qu'il en est de même de celles de Beams, car nous avons observé bien souvent, dans diverses cellules animales par des colorations vitales au rouge neutre, des vacuoles tout à fait semblables à celles que l'on trouve dans les cellules embryonnaires des Végétaux et qui ne sauraient être considérées comme provoquées par le colorant, ce qui confirme les résultats, d'ailleurs très solidement établis, de Parat et d'autres auteurs.

#### Index Bibliographique

- 1) Eliot Weier (T)—Neutral red staining in the protonema of *Polytrichum commune*. Amer. Jour. Bot. 20, 341-439, June 1933.
- 2) Guillaiermond (A)—Sur le développement d'un *Saprolegnia* en milieu additionné de colorants vitaux. C. R. Ac. Sc. 189, 1261, 1929. Germination des zoospores et formation des zoosporanges dans un *Saprolegnia* en culture sur milieux additionnés de rouge neutre. C. R. Ac. Sc. 190, 384, 1930.
- 3) ——— Nouvelles recherches sur la coloration vitale par le rouge neutre dans les cellules végétales. C. R. Ac. Sc. 188, 813, 1929. Le vacuome des cellules végétales. Protoplasma, 9, 133, 1930.
- , Dufrénoy (J) et Labrousse—Germination des graines de Tabac en milieux additionnés de rouge neutre et coloration vitale de leur vacuome pendant la croissance. C. R. Ac. Sc. 190, 1439, 1930.
- et Obaton (F)—Sur l'action du pH sur la coloration vitale dans les cellules végétales. C. R. Soc. Biol. 114, 805, 1933.
- Reilhaes (R), Stérides et phospholipides dans le système vacuolaire de la cellule végétale. Thèse de Doctorat ès Sciences. Nov. 1936—Rev. de Cytologie et de Cytophysologie végétale.

## Chromosome Studies in Cyperaceae, I<sup>1)</sup>

By

**Nobunori Tanaka**

Tokyo Imperial University

From the cytological point of view the Cyperaceae present several specific features among Angiosperms. One of these features is found in the development of their pollen grains. The pollen mother cell undergoes a meiotic division usually which results in four tetrad nuclei of which only one develops to become a normal pollen grain, while the remaining three degenerate (11). In the pollen grain generative and vegetative nuclei are produced by the primary nuclear division (cf. 1, 14, 15, 16). Another striking feature lies in the chromosome numbers. Thanks to Heilborn, Håkansson, Hicks and others the chromosome numbers in this family have become widely known. However, it has been worthy of notice, from the view points of chromosome evolution or species formation, that the numbers found in this family were not generally multiples of any basic numbers. The cause and nature of this phenomenon have been considered and discussed from different quarters by many authors but seem to remain as yet unsettled and require adequate explanations based on further observations. In Japan plenty of Cyperacean plants are found most of which have not been submitted to cytological investigations. On this occasion a preliminary report is made on the chromosome numbers of 19 species and 3 varieties.

### Materials and Methods

Materials were taken from the plants grown either cultivated or wild in the Koisikawa Botanic Garden of Tokyo Imperial University, though some were collected in other localities. The inflorescences were fixed with Carnoy's fluid for several hours. After being washed in 95% alcohol, they were preserved in 75% alcohol until necessary.

From the preserved inflorescences the anther sacs were taken out with needles under the magnifying-glass. After addition of a drop of aceto-carmin, they were covered with a cover glass on which a slight pressure was applied with the tip of the needle. The cover

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glass was sealed with paraffin. The preparations thus obtained could be used for observation over a period of 3 weeks.

All camera-lucida drawings were made from such aceto-carminic preparations, magnifications being  $\times 1600$  and  $\times 1300$  reduced one half in reproduction.

### Observations

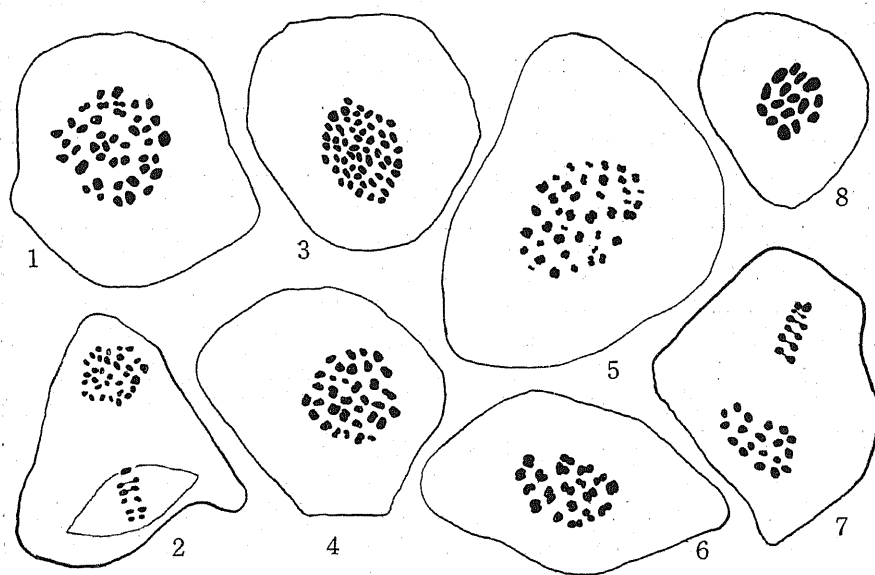
#### *Bulbostylis*

Concerning this genus no cytological investigations have hitherto been made so far as the writer is aware.

*Bulbostylis capillaris* Kunth. var. *trifida* Clarke was collected at Karuizawa, Nagano Prefecture and near Yamanaka-ko, Yamanashi Prefecture. Forty-two chromosomes which show some difference in their size (Fig. 1) were counted in the first meiotic metaphase of this plant.

#### *Carex*

As regards the genus Heilborn (1922, 1924, 1925, 1932, 1936) published a series of studies on the chromosomes, their numbers and size, polyploidy and aneuploidy, the so-called secondary association and also species formations in this genus. He has investigated about 70 species and forms, and found some 27 different numbers, ranging



Figs. 1-8. First and second metaphases of *Bulbostylis*, *Carex* and *Cyperus*.  $\times 1600$ .  
 1, *B. capillaris* var. *trifida* ( $n = 42$ ). 2, *C. japonica* var. *chlorostachys* ( $n = 31$ ).  
 3, *C. neurocarpa* ( $n = 54$ ). 4, *C. Maackii* ( $n = 34$ ). 5, *C. pumila* ( $n = 41$ ). 6, 7, *C. satsumensis* ( $n = 19$ ). 8, *Cyp. alternifolius* ( $n = 16$ ).

from 9 to 56 which do not come under the law of multiples of any basic numbers, namely, 9, 15, 16, 18, 19, every number of 23-43, and 56 (1924, 1928, 1932). Wulff (1933) reported the haploid number of one species as 30-32. Such *Carex*-type of aneuploidy has been met with among other genera of Cyperaceae as well.

In the present work four species and one variety of this genus were investigated.

*Carex japonica* Thunb. var. *chlorostachys* Kuek.  $n = 31$ , Fig. 2; *C. neurocarpa* Maxim.  $n = 54$ , Fig. 3; *C. Maackii* Maxim.  $n = 34$ , Fig. 4; *C. pumila* Thunb.  $n = 41$ , Fig. 5; *C. satsumensis* Franch. et Sav.  $n = 19$ , Figs. 6, 7.

In *Carex neurocarpa* Maxim., a considerable size difference exists among chromosomes seen in the heterotypic metaphase plate. *C. satsumensis* was collected at Mt. Ôyama, Sagami Province or at Nikkô, Totigi Prefecture. In the heterotypic metaphase 19 chromosomes are counted, of which five are somewhat smaller than the rest. The haploid number 54 of *C. neurocarpa* is added to the number series of this genus, resulting in 9, 15, 16, 18, 19, every number of 23-43, 54 and 56.

### *Cyperus*

In *Cyperus*, also, aneuploid numbers were found by Hicks (1929), Avdulov (1931) and Lewitsky (1931), that is, 13, 17, 21, ca. 25, 48, 54, 73.

In the present work six species of this genus were investigated.

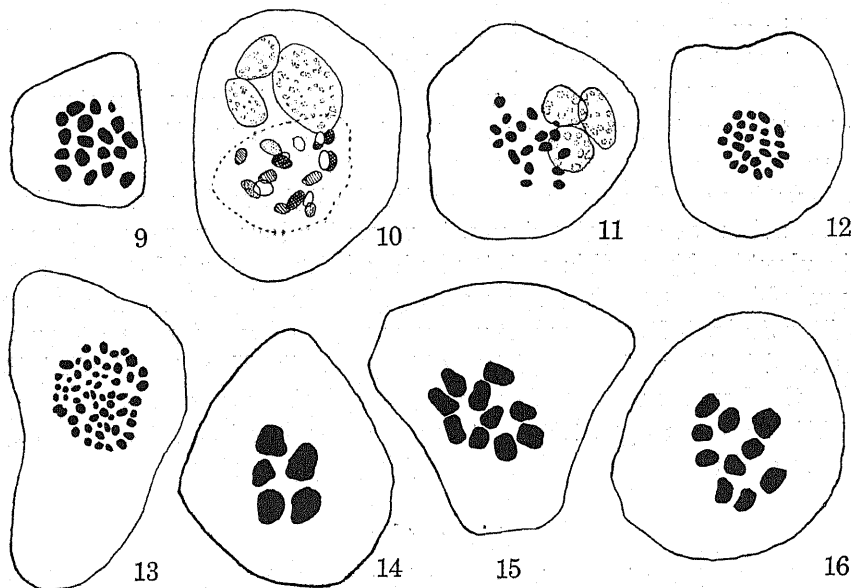
*Cyp. alternifolius* L. from Madascaris is cultivated in the green house of the Koisikawa Botanic Garden of Tokyo Imperial University. In the heterotypic metaphase plate of the pollen mother cells 16 chromosomes were counted (Fig. 8).

*Cyp. hakonensis* Franch. et Sav.  $n = 18$ , Figs. 9-11. Eighteen chromosomes were counted in the heterotypic metaphase and in the metaphase of the primary nuclear division in the pollen grain. One of them is conspicuously smaller than the others. The three degenerating nuclei of the tetrad are shown in Figs. 10 and 11.

*Cyp. sanguinolentus* Vahl ( $n = 24$ , Fig. 12) was collected at Karuizawa, Nagano Prefecture. Fig. 12 shows a heterotypic metaphase plate. All stages of meiotic division are regular.

*Cyp. rotundus* L. ( $n = 54$ , Fig. 13) collected at Kanazawa, Kanagawa Prefecture, was examined. Fig. 13 presents a heterotypic metaphase plate in which the chromosomes show a size difference. It is however difficult to determine the exact groups in this respect, owing to the smallness of the chromosomes as in the case of the genus *Cyperus* in general. In the following species the minuteness and a

rather high number of the chromosomes made the exact counting difficult. In *Cyp. nipponicus* Franch. et Sav. several countings are obtained such as 76, 82, 83 and 87. In *Cyp. papyrus* L. about 51 chromosomes were found in the heterotypic metaphase.



Figs. 9-16. First and second metaphases of *Cyperus*, *Fimbristylis* and *Heleocharis*.  $\times 1300$ . 9-11, *Cyp. halconensis* ( $n = 18$ ), 9, first metaphase; 10, 11, late diakinesis and metaphase of the primary nuclear division in the pollen grain, 12, *Cyp. sanguinolentus* ( $n = 24$ ). 13, *Cyp. rotundus* ( $n = 54$ ). 14, *F. sub-bispicata* ( $n = 5$ ). 15, *H. acicularis* ( $n = 10$ ). 16, *H. japonica* ( $n = 10$ ).

### *Fimbristylis*

No cytological studies have previously been made on this genus.

*Fimbristylis sub-bispicata* Nees et Mey. ( $n = 5$ , Fig. 14) was collected at the foot of the Mt. Tatesina in Nagano Prefecture and at Kanazawa in Kanagawa Prefecture. The haploid number is 5 of which one is smaller than the others.

### *Heleocharis*

*Heleocharis acicularis* R. Br. ( $n = 10$ , Fig. 15) was grown wild at Karuizawa, Nagano Prefecture. The inflorescences suitable for cytological studies are about 1.5-2 mm long by 0.7-1 mm wide. The heterotypic metaphase shows 10 bivalent chromosomes, of which three are rather smaller than the others.

*H. japonica* Miq. ( $n = 10$ , Fig. 16) was also collected at Karuizawa, Nagano Prefecture. The size of the inflorescences suitable for

cytological studies are about 2–2.5 mm long by 1–1.2 mm wide. Ten chromosomes were counted in the heterotypic metaphase plate.

In *Heleocharis* (= *Eleocharis*) Hicks (1929) has reported 8 aneuploid chromosome numbers, 5, 8, 8–9, 10, 15, 18, 19, 25–29. Studying *H. acicularis* (L.) R. & S. Hicks (1929) reported several haploid numbers, 18, 19, 28, 25–29. He states, "The late prophase showed 28 chromosomes in the majority of cases. In the heterotypic metaphase plate the number varied 25–29. In the second metaphase plates the number varied as 18, 19 etc.... The peculiarities in the union of homologous chromosomes, unstable conditions in pairing, and lagging of chromosomes seem, in the light of known conditions of the origin of such structures, to mark the heterozygous nature of this plant." From this point of view Hicks offered hybridism as a probable explanation of the aneuploidy found in the Cyperaceae. In *H. acicularis* R. Br., in the present work, no such irregularity was observed in the meiotic division (Fig. 15). In *H. palustris* (L.) R. & S., the haploid number is also varied; that is, according to Hicks (1929) they are 8, 9, 18 and  $2n = 9$ , according to Avdulof (1931) it is 8, and according to Lewitsky (1934) 5 and 8.

### *Lipocarpus*

*Lipocarpus microcephala* Kunth.  $n = 23$ , Fig. 17. The heterotypic metaphase shows 23 chromosomes which may be divided into three size groups: 2 large, 17 medium and 4 small.

No other species or varieties of this genus have hitherto been investigated.

### *Scirpus*

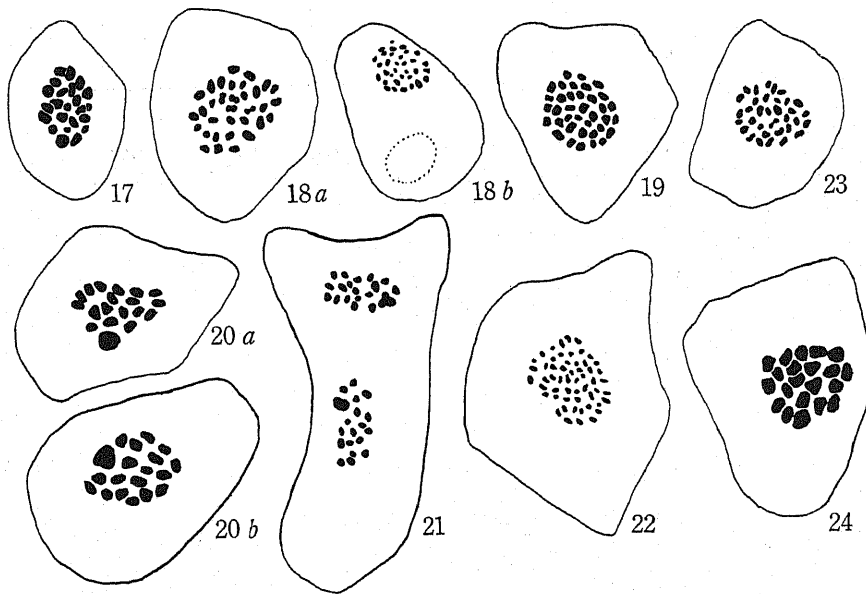
In the genus *Scirpus*, Håkansson (1928, 1930), Hicks (1928), Avdulof (1931), Blackburn (1933), Kostrioukoff (1930) and Morinaga & Fukushima (1931) have reported the chromosome numbers, 10, 13, 18, 19, 20, 21, 22, 23, 25–30, 28, 29, 31, 33, 34, 38, 39, 43, 50–60, 52, 53–55, 55, 55–57.

*S. cyperinus* Kunth. var. *Wichurii* Makino.  $n = 33$ , Figs. 18 a, b. The haploid chromosome number of this variety agrees with that found by Hicks (1928) in a variety of this species, *pelius* Fern. and in the forma *condensatus* (Fern.) Blake of this variety.

*S. hotarui* Ohwi ( $n = 37$ , Fig. 19) is one of 6 species which were recently established by Ohwi (1934). These new 6 species have hitherto been grouped into one species *Scirpus erectus* Poir. .

*S. lacustris* L. ( $n = 19$ , Figs. 20a, b, 21, 25, 26, 27) has 19 haploid chromosomes, of which one element is conspicuously larger than the rest. This large element is massy or sometimes deltoid in shape in

the first metaphase, while in the second metaphase its deltoid nature becomes more evident (Figs. 21, 26). Studying this species also Håkansson (1928) reported 21 haploid chromosomes which however showed no such size difference as seen in the present case, though there were found three or four somewhat larger chromosomes. It is probable that the large element in the present material is composed of three chromosomes.



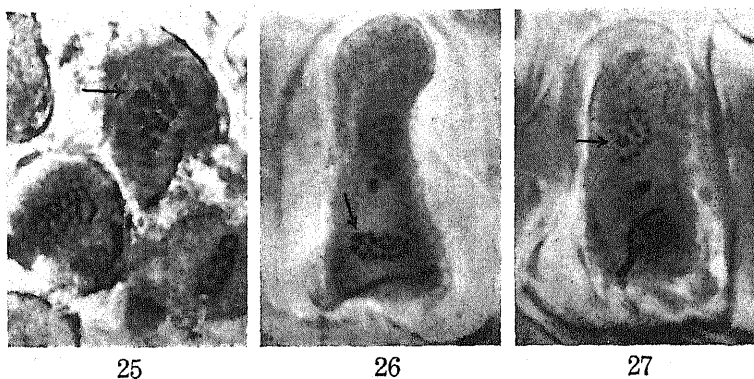
Figs. 17-24. First and second metaphases of *Lipocarpha* and *Scirpus*.  $\times 1600$ , except 18a, b., 22, 23,  $\times 1800$ . 17, *L. microcephala* ( $n = 23$ ). 18a, b. *S. cyperinus* var. *Wichurii* ( $n = 33$ ). 19, *S. hotarui* ( $n = 37$ ). 20a, b., 21, *S. lacustris* ( $n = 19$ ). 20, first metaphase. 21, second metaphase. 22, *S. maritimus* ( $n = 55$ ). 23, *S. Mitsukuriana* ( $n = 34$ ). 24, *S. mucronatus* ( $n = 21$ ).

*S. maritimus* L.  $n = 55$ , Fig. 22. The chromosomes are very small which makes the counting very difficult. Fifty-five seems most probable, 2 of which are conspicuously smaller than the others. These small ones are sometimes lying close together and sometimes widely apart. The chromosome number of this species was formerly reported as ca. 52 by Håkansson (1928) and as 43 by Blackburn (1933).

*S. Mitsukuriana* Makino  $n = 34$ , Fig. 23.

*S. mucronatus* L.  $n = 21$ , Fig. 24. This haploid number agrees with that reported by Morinaga and Fukushima (1931).

In *Scirpus*, Håkansson (1930) has found two races of *S. palustris*, one with 19 chromosomes (haploid) and one with 8. He stated that the former appears to be a derivated autotetraploid.



Figs. 25-27. Photomicrographs of the first and second metaphases of *Scirpus lacustris*, corresponding to Figs. 20a, b, and 21 respectively (the large element is marked with an arrow).  $\times 1300$ .

In *S. americanus* Pers. and its form, Hicks (1928) found different haploid numbers, the former 38 and the latter 50-64. He considered this latter form with varying chromosome numbers might have arisen through crossing of the former with some other species.

### Conclusion and Summary

A number of possible explanations have been presented by several authors on the phenomena of aneuploidy observed in Cyperaceae: 1) transverse division of single (large) chromosome (Heilborn, Håkansson), 2) duplication of entire chromosomes (Heilborn, Håkansson) and 3) hybridization (Hicks, Håkansson). In the present work in which the meiotic chromosome numbers of 19 species and 3 varieties were described, aneuploidy has also been observed. It is however premature to draw any conclusion as to the cause and nature of this phenomenon until further investigations on a greater number of plants have been carried out.

The numbers of chromosomes in three genera of Cyperaceae, *Bulbostylis* ( $n = 42$ ), *Fimbristylis* ( $n = 5$ ) and *Lipocarpus* ( $n = 23$ ), have been newly described.

The haploid number 54 (*Carex neurocarpa*) is newly added to the series of chromosome numbers of the genus *Carex*.

The present material of *Heleocharis acicularis* has  $n = 10$  and shows no irregularity in the chromosome number as observed by Hicks in the plant he was using.

According to Håkansson, *Scirpus lacustris* has  $n = 21$  chromosomes, while in the present material one large element was found besides 18 normal bivalent chromosomes making 19 in all. This large element seems to be composed of three bivalents.



Most of the plants used in the present work were identified by Dr. Hara, Dr. Tuyama, Dr. Kitagawa and Dr. Maekawa to whom the writer expresses his cordial thanks. Thanks are due to Dr. Sinotô under whose direction the work has been carried out.

Division of Genetics, Botanical Institute,  
Faculty of Science, Tokyo Imp. University

#### Literature

1. Håkansson, A. 1927-1928. Die Chromosomen einiger Scirpoideen. *Hereditas* 10: 277-292.
2. — 1930. Über verschiedene Chromosomenzahlen in *Scirpus palustris* L. *ib.* 13: 53-60.
3. Heilborn, O. 1918. Zur Embryologie und Zytologie einiger *Carex*-Arten. *Sv. Bot. Tids.* 12: 212-220.
4. — 1922. Die Chromosomenzahlen der Gattung *Carex* (Vorläufige Mitteilung). *ib.* 16: 271-274.
5. — 1924. Chromosome numbers and dimensions, species-formation and phylogeny in the genus *Carex*. *Hereditas* 5: 192-216.
6. — 1925. Genetic cytology and genetics in *Carex*. *Bibl. Genet.* 1: 459-461.
7. — 1932. Aneuploidy and polyploidy. *Sv. Bot. Tids.* 26: 137-146.
8. — 1936. The mechanics of so-called secondary association between chromosomes. *Hereditas* 22: 167-188.
9. Hicks, G. 1928. Chromosome studies in the Cyperaceae with special reference to *Scirpus*. *Bot. Gaz.* 86: 295-317.
10. — 1929. Cytological studies in *Cyperus*, *Eleocharis*, *Dulichium* and *Eriophorum*. *ib.* 88: 132-149.
11. Juel, H. 1900. Beiträge zur Kenntnis der Tetradenteilung. *Jahrb. f. wiss. Bot.* 35: 626-659.
12. Morinaga, T. and Fukushima, E. 1931. Chromosome numbers of cultivated plants III. *Bot. Mag. (Tokyo)* 45: 140-145.
13. Ohwi, J. 1934. Notes on Cyperaceae IV. *Acta Phytotaxon. et Geobot.* 3: 165-167.
14. Piech, K. 1924. Über die Teilung des primären Pollenkerns und die Entstehung der Spormazellen bei *Scirpus palustris* L. *Bull. l'Acad. Polon. Sci. et Lettr. Cl. Sc. Math. Nat. Ser. B*: 605-621.
15. — 1928a. Zytologische Studien an der Gattung *Scirpus*. *ib.* (1928): 1-43.
16. — 1928b. Über die Entstehung der generativen Zelle bei *Scirpus uniglumis* Link. durch freie Zellbildung. *Planta* 6: 96-117.
17. Tischler, G. 1935-1936. Pflanzliche Chromosomen-Zahlen. *Tab. Biolog.* 11, 12.

## Macrosporogenesis of *Lilium philippinense* Baker

By

José K. Santos

Of the Department of Botany, University of the Philippines

The object of this paper is to describe the development of the embryo-sac of *Lilium philippinense* Baker, with a view to its possible bearing on the recent interpretations regarding the increase in size and the increase in the chromosome number of the chalazal nuclei following the homoeotypic division in the lily type of embryo-sac.

Treub and Mellink (20) in 1880 reported that in *Lilium bulbiferum* and *Tulipa Gesneriana* the embryo-sac mother cell develops directly into the embryo-sac without any previous divisions.

In 1884 Strasburger (18) and Guignard (9) came to the same conclusion in their separate investigations of a reduction of the number of chromosomes during the development of the germ cells of angiosperms.

Guignard (11) and Overton (13), whose works were published almost at the same time, concluded that in the lilies and other plants in which the embryo-sac mother cell develops directly into the embryo-sac without previous division reduction takes place in the nucleus of the young embryo-sac.

Guignard (9) working on the embryo-sac of *Lilium candidum* observed the difference in size and the difference in number of chromosomes between the nucleus near the micropyle and the one close to the chalazal end; the micropylar nucleus is slightly smaller and has fewer chromosomes than the chalazal nucleus.

Dixon (7) reported that in *Lilium longiflorum* the superior nucleus of one embryo-sac contains twelve chromosomes while the inferior nucleus has twenty-four and in another embryo-sac contains eight chromosomes whereas the inferior one has sixteen chromosomes.

Sargant (15) noticed the same differences in the embryo-sac of *Lilium martagon*. She counted twelve chromosomes in the micropylar nucleus and about twenty-four in the chalazal one. A further count made from the chalazal nuclei she obtained among the twenty-five chalazal spindles in which the chromosomes could be approximately counted, one had about twenty, nine about twenty-four, five about twenty-eight, and ten about thirty-two chromosomes.

Similar irregularities were described in the embryo-sac of *Lilium martagon* by Mottier (12) and also by Strasburger (17).

Coulter (5) noticed the same in *Lilium philadelphicum* and Strasburger (19) in *Tulipa Gesneriana*.

Bambacioni (1) reported that in *Fritillaria persica* three of the four nuclei resulting from the homoeotypic division of the megaspore mother cell come to lie close together in the chalazal end of the embryo-sac and then divide simultaneously, their chromosomes become arranged on a common spindle. As a result of this division, a four-nucleate embryo-sac is again formed, two of whose nuclei are small and lie near its micropylar end, and the other two are larger and have a greater number of chromosomes at the chalazal end.

A type of embryo-sac development similar to that of *Fritillaria persica* was reported later by Bambacioni and Giombini (2) in *Tulipa Gesneriana*, another by Bambacioni-Mezzetti (3) in *Lilium candidum*, *L. bulbiferum*, and *Tulipa praecox*, and recently by Cooper (4) in *Lilium Henryi*.

### Material and Methods

This work was started during the academic year, 1934-35 while the writer was connected with the Department of Botany, University of Michigan, U. S. A., as an exchange professor in botany.

Part of the material used was generously furnished by Professor Bradley M. Davis to whom the writer is highly indebted. This material was already embedded in paraffin and was prepared from the *Lilium philippinense* grown in their garden. The material, however, showed very few chromatic figures so the investigation was discontinued until the writer returned to Manila, and after securing additional material from Baguio, the natural habitat of this plant.

The additional material, upon which the most of the observations were based, was furnished by Mr. Sixto Laraya, district forester at Baguio, Mountain Province, Luzon, to whom the writer is very grateful. This material was collected from the slopes of a mountain at an altitude of about 5,000 feet above sea-level, and immediately forwarded to the writer in Manila. He sent to the writer two sets of material, the first set consisting of fifteen plants with young buds and bulbs, which was received June 26, 1936; and the second set, composed of fifty plants with young buds and nearly opened flowers, was received July 6, last.

The ovaries were sliced to about 3 millimeters thick and fixed between 2 and 3 o'clock in the afternoon with the following reagents: (1) Weak Flemming's fluid, (2) Strong Flemming's fluid, and (3) 1 per cent chromo-acetic-acid, with about 10 drops of 1 per cent osmic acid to 50 cubic centimeters of the solution.

The fixed material was thoroughly washed with running water, dehydrated and cleared in the usual way, and embedded in 52°C. paraffin. It was cut from 8 to 20  $\mu$  thick and stained in Heidenhain's iron-alum haematoxylin.

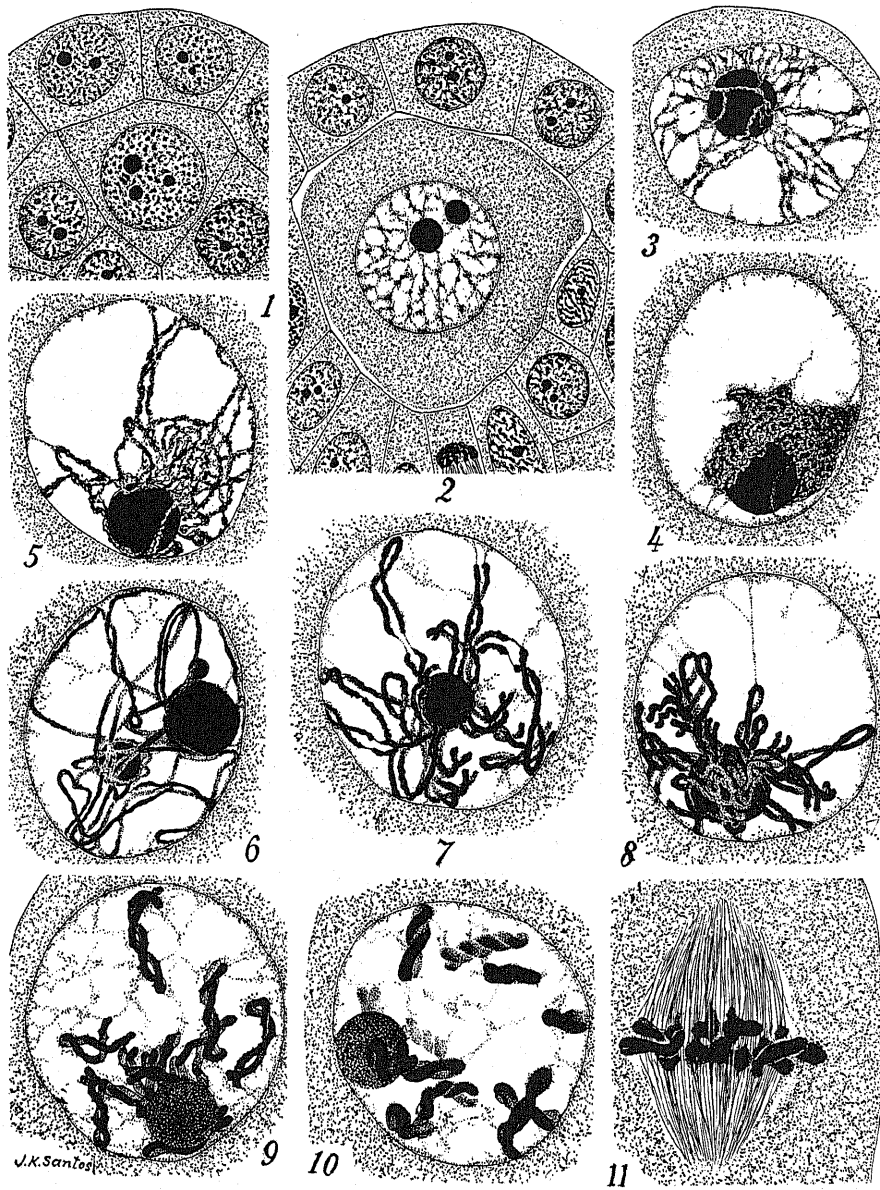
### Observations

The megasporocyte of *Lilium philippinense* Baker is developed by the enlargement of a subepidermal cell of the nucellus. This cell is differentiated from the nucellar cells surrounding it, immediately after the nucellus has prominently protruded from the placenta, and some time before the development of the integuments is initiated. The young megasporocyte appears usually triangular in outline and some times polygonal with unequal sides and with a fairly large nucellus as represent in fig. 1. The nucleus remains for some time in the resting stage. Its structure before further enlargement takes place is quite similar to that of the nucellar nuclei surrounding it. The chromatin is uniformly distributed over the cavity of the nucleus in the form of a network with extremely small meshes. Its general appearance is dark and granular on account of the tiny dots or beadlike structure of the chromatin at the intersections of the threads. There is either a single large nucleolus or, more commonly, three or four of different sizes concealed in the dotted chromatin threads. The cytoplasm is very dense and granular in character.

As the megasporocyte nucleus increases in size the meshes become larger, the threads and the dots acquire more distinct outlines. The appearance of the nuclear cavity is much lighter than at the earlier stages and the nucleoli are more conspicuous. Fig. 2 represents a resting stage in which a maximum enlargement before the nucleus passes into prophase is shown. The reticulum shows large and somewhat uniform meshes. The threads and the chromatin dots or beads are more sharply defined and occupy mostly the peripheral part of the nuclear cavity. At this stage the development of the integuments is initiated. One cell on each side of the nucellus begins to divide and protrude outward in the form of a hump.

As the nucleus continues enlarging the reticulum loses its uniform reticular character, the chromatin dots or beads become larger and closer together, and the threads become thicker. Some of the meshes become larger and others become smaller and disappear; thus the reticulum acquires a ragged appearance. Fig. 3 illustrates the first indication of the megasporocyte nucleus proceeding to synizesis. The dots are larger and the threads are much thicker. As the nucleus proceeds to synizesis the reticulum gradually separates from the nuclear wall from one side of the nuclear cavity or sometimes from almost all around the nuclear membrane. While

the contraction of the reticulum advances, the nucleus grows in size. The contraction continues until all the threads of the reticulum are



Figs. 1-11. 1. A young megasporocyte surrounded by nucellar cells. 2. Resting stage of megasporocyte nucleus. 3. The megaspore mother cells passing in synizesis. 4. Synizesis. 5. A young spireme loosening itself from synizetic knot. 6. Open spireme. 7. Segmentation. 8. Second contraction or synapsis. 9. Young bivalent chromosome coming out from synapsis. 10. Diakinesis. 11. Heterotypic metaphase. All figures 1-11 magnified  $\times 925$ .

drawn into a compact and characteristic mass at one side of the nucleus, inclosing partly or entirely the nucleolus (fig. 4). The nucleus remains in this stage for some time. While the nucleus is in the synizetic stage, the microsporocytes elongates gradually and the integuments develop more rapidly.

Following the long and critical state of the chromatin reticulum in synizesis, a continuous and irregularly thickened thread with ragged appearance emerges from the side of the synizetic knot. A careful examination of the spireme reveals that it is made up of two rows of closely arranged chromatin granules similar to what Sargant (15) reported in *Lilium martagon*, Digby (6) in *Osmunda*, and the writer (14) in *Elodea*. Fig. 5 represents a stage in which the young spireme is loosening itself from the synizetic knot. As the spireme loosens itself from the synizetic knot loops of various sizes radiate from the knot to the sides of the nuclear cavity and the nucleolus or nucleoli are released from their entanglement with the spireme. Subsequent to this stage, as usual, the spireme passes into the process of shortening and condensation. Fig. 6 represents a more advanced stage of the spireme. The spireme appears more uniformly in diameter and takes up the stain more evenly. Its double nature disappears. As the condensation, shortening, and thickening of the spireme continue the loops rearrange themselves in a very irregular manner and the sides of each of them are brought closer to one another. The loops are gradually separated by constriction or segmentation from each other, as shown in fig. 7. One notable feature of this stage is that the arms or sides of the loops become crooked and in most cases are twisted around each other. A very careful examination of the individual loop revealed that there is a constriction at their distal end which apparently indicates that each side of each loop represents a univalent chromosome. As the segmentation advances, the segments are collected or become entangled with each other at one side or around the nucleolus. Figure 8 shows a typical second contraction in the megasprocytes in *Lilium philippinense*.

The young conjugating chromosomes remain in this state of synapsis for a short period only and soon the folded segments distribute themselves gradually throughout the nuclear cavity as represented in fig. 9. The two arms of the loops can be readily traced; they are still slender and irregularly thickened and as a whole are twisted around each other. Constriction at the distal end of each young bivalent chromosome is observed, while the double nature of the sides of the original is not conspicuous. As usual soon after the young bivalent chromosomes are evenly distributed in the nuclear

cavity, they undergo a more extensive condensation, shortening, and thickening. Fig. 10 represents a more-advanced diakinetik stage in which the bivalent chromosomes appear much thicker and shorter, and the members of each of them are tightly twisted around each other. Starting from this stage, careful counts of the chromosomes were made. Various counts were made from a number of nuclei, and the number obtained in each nucleus was always twelve. This haploid number agrees quite well with the counts obtained from the diploid number obtained from the various counts made from the dividing nuclei of the nucellus, which is twenty-four.

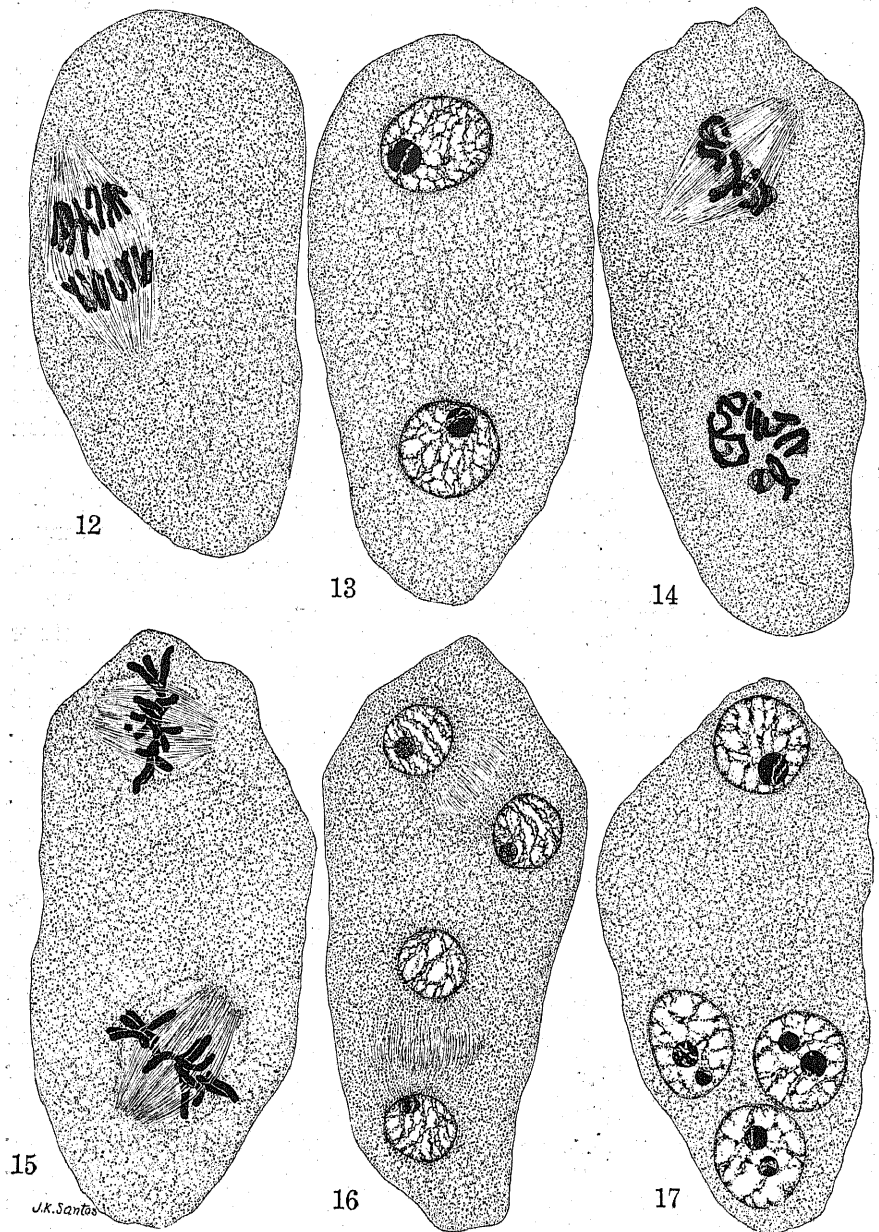
While the chromatin segments proceed into second contraction or synapsis the nucleolus begins to decrease in size and becomes vacuolated and eventually acquires a lighter color. It disappears at the same time with the nuclear membrane during the formation of the spindle fibers.

As soon as the spindle fibers appear in the nuclear cavity, the bivalent chromosomes, which by this time have attained a maximum shortening and condensation, become attached to the spindle fibers either at the middle part or near one end. The chromosomes move to the equatorial plane to form the equatorial plate. At this stage the longitudinal fission of each chromosome can sometimes be observed. Figure 11 illustrates the arrangement of the bivalent chromosomes in metaphase stage. The two members of each chromosome are attached either at the middle part or close to their ends by small group of spindle fibers. The members of each bivalent chromosome separate gradually and move away from the equatorial region towards the poles. As they migrate towards the respective poles they appear, according to the point of attachment with the spindles, either J- or V-shaped as represented in fig. 12.

Shortly after the daughter chromosomes reach the respective poles, they are collected in the form of a compact mass and gradually surrounded by a dense protoplasm followed by the formation of nuclear membrane. Immediately upon the formation of the nuclear membrane, the chromosomes begin to loosen themselves from one another while the nuclear cavity increases in size and the corresponding nucleoli simultaneously appear. The chromosomes become connected with each other by fine strands and develop into more or less uniform reticuli. The resulting daughter nuclei are of the same size, and they are not separated by a wall. Fig. 13 shows the two daughter nuclei resulting from the first division in a resting stage.

After a short period of rest, the chromatin begins to reorganize and prepare for the second division. The chromatin threads developed in the usual way into spireme which give rise to chromo-



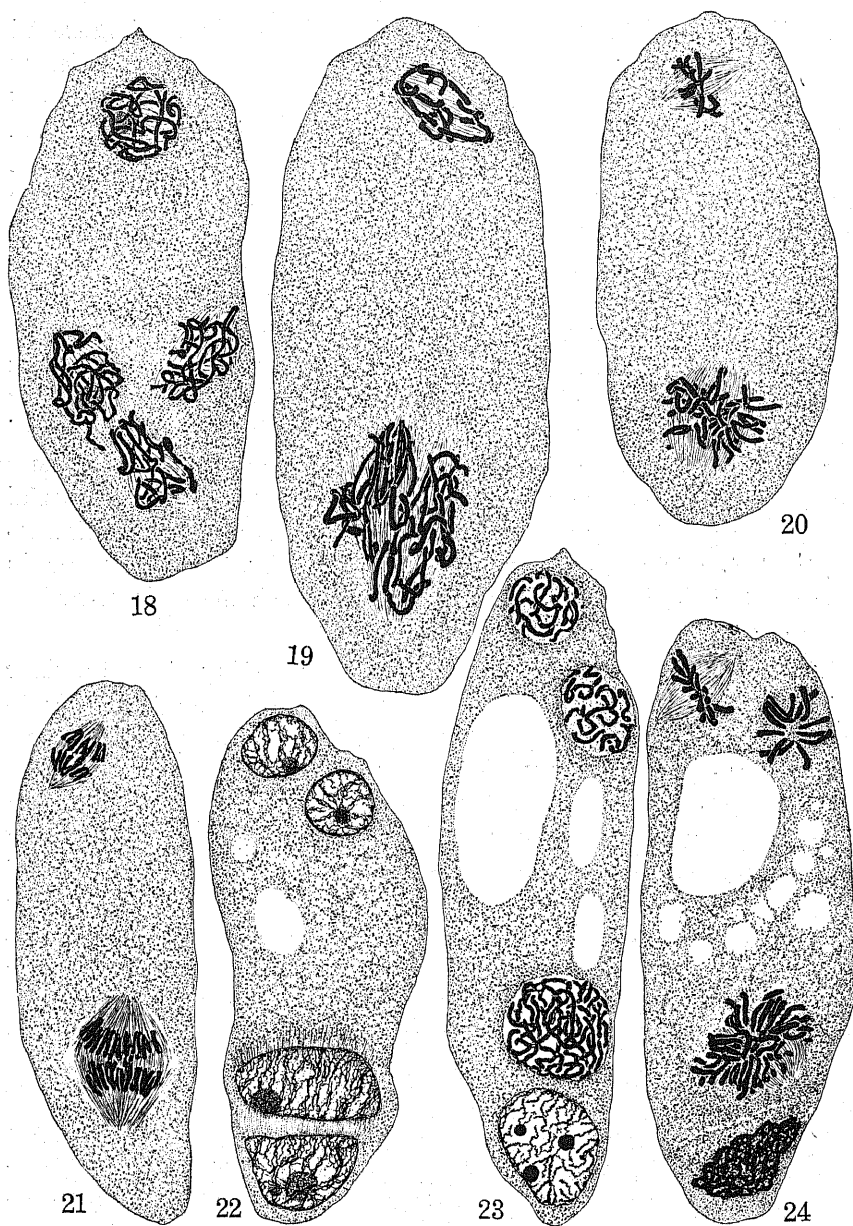


Figs. 12-17. 12. Heterotypic anaphase. 13. The micropylar and chalazal daughter nuclei in the resting stage. 14. Early metaphase of the homoeotypic division; the micropylar nucleus at the side view and the chalazal nucleus in polar view. 15. Side view of the metaphase of the homoeotypic division. 16. Late telophase of the megasporial nuclei. 17. Resting stage of the four megasporial nuclei. All figures 12-17 magnified  $\times 625$ .



somes that later become attached to the spindle fibers. Figure 14 shows the chromosomes developed from the two daughter nuclei as they reached their respective equatorial planes. The chromosomes towards the micropylar end are seen in the side view of the spindle, while those toward the chalazal end are observed from the polar view. Figure 15 illustrates a normal metaphase stage of the two daughter nuclei, which divide homoeotypically without cell division, forming four megasporial nuclei in the embryo-sac. Figure 16 shows the four young megasporial nuclei under the process of reconstruction and enlargement. The spindle fibers between the two nuclei of each pair are still visible and show no sign of wall formation. One of the four megasporial nuclei resulting from the homoeotypic division moves slightly towards the micropylar end, while the three nuclei pass to the chalazal extremity, arranging themselves in a close triangular form as indicated in figure 17. These four megasporial nuclei have practically the same size from the time of their formation to their adult or resting stage (Plate 30, fig. 1). They remain for some time in the resting stage, during which the embryo-sac increases extensively in length.

Following the polarization and the long resting stage the four megasporial nuclei, which are all alike, proceed simultaneously to the third division (Plate 30, fig. 2). Upon the formation of the chromosomes, the nucleoli and the nuclear membrane disappear, while the spindle fibers appear almost at the same time as represented in fig. 18. In the following stage the twelve chromosomes of the megasporial nucleus around the micropylar end pass to the equatorial plane in a bipolar spindle, while the spindle fibers of the thirty-six chromosomes of the three megasporial nuclei become united in a multipolar spindle, which in the later development becomes bipolar as indicated in fig. 19 and plate 30, fig. 3. In fig. 20 the multiple spindle has become bipolar and the thirty-six chromosomes are arranging themselves in the equatorial region of the spindle. It usually takes much longer for the thirty-six chromosomes to arrange themselves in the equatorial plane of the spindle than the twelve chromosomes of the nucleus near the micropyle. However, it seems that the two groups of chromosomes divide simultaneously as indicated in fig. 21, because the distance between the resulting daughter chromosomes of the division is about the same. In view of this unusual division a four-nucleate embryo-sac is again formed. Two of the resulting nuclei are smaller and remain near the micropylar end, and two are larger and lie at the chalazal end as shown in fig. 22. As the upper chalazal nucleus advances into the resting stage, it becomes flattened or lenticular, extending from one side to



Figs. 18-24. 18. The four megasporial nuclei passing into third division; the spindles of the three chalazal nuclei are developing.  $\times 625$ . 19. The chromosomes of the micropylar nucleus begins to move to the equatorial plane of the spindle, while the chromosomes and the spindles of the three chalazal nuclei become united.  $\times 625$ . 20. Early metaphase of the micropylar and chalazal nuclei.  $\times 450$ . 21. Anaphase in the third division.  $\times 450$ . 22. A four-nucleate embryo-sac showing two small micropylar nuclei and two large chalazal nuclei.  $\times 450$ . 23. Early stage of the fourth division showing the lower chalazal nucleus lagging.  $\times 450$ . 24. Metaphase of the fourth division. The lower chalazal nucleus in spireme stage.  $\times 450$ .

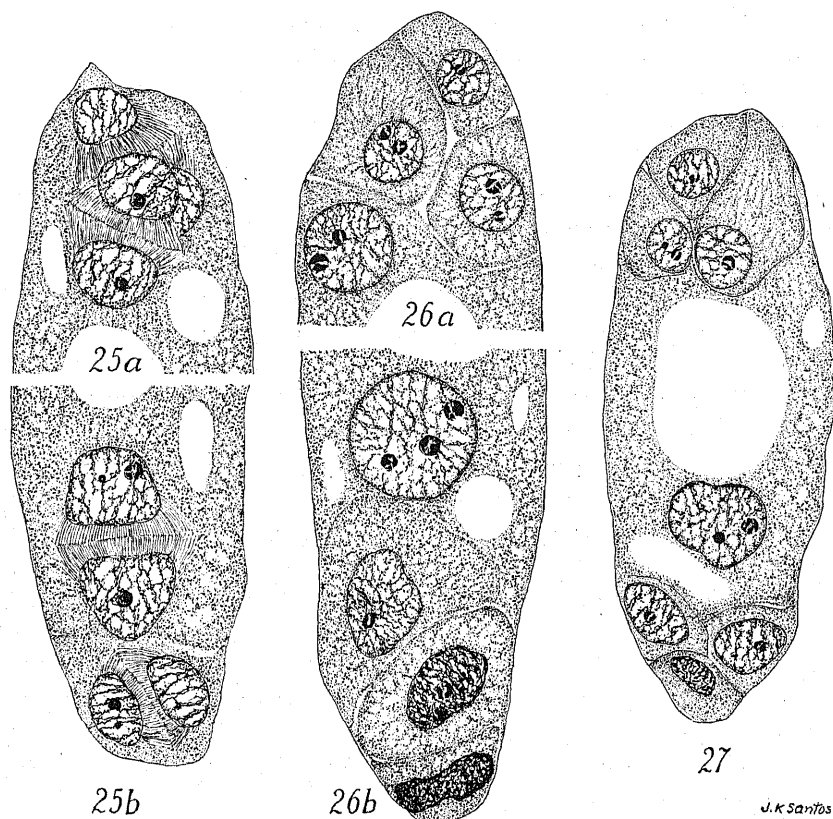
other of the embryo-sac; and the lower chalazal nucleus usually acquires the shape of the end of the embryo-sac. It appears like the side view of a mortar or tea cup. These two nuclei usually remain somewhat connected by the spindles until before the last division.

One of the notable change observed in the embryo-sac up to this stage was that the cytoplasm, which had been dense and uniform before the formation of the two pairs of unequal-sized nuclei, became somewhat ragged in appearance and two vacuoles appeared in the central region between the two groups of nuclei at the two extremities. As the four nuclei proceed to another division to form the eight nuclei, the embryo-sac increases rapidly in size and the vacuoles either coalesce or increase proportionately in dimensions which separates the two groups of nuclei.

Fig. 23 shows the four nuclei are proceeding to the fourth division. The two megasporial nuclei, just before the formation of the spindle, show twelve chromosomes each. The upper nucleus in the chalazal region exhibits a large number of chromosomes or the thirty-six chromosomes resulting from the combination of the chromosomes of the three megasporial nuclei. It is about the same stage as the micropylar nuclei, while the lower chalazal nucleus is still in the spireme stage or segmentation. At this stage several counts were made in the upper chalazal nucleus and the number obtained varies from thirty-two to thirty-six chromosomes. Shortly after the spindle fibers are developed, the chromosomes of the two micropylar nuclei and those of the upper chalazal nucleus arrange themselves in the equatorial regions of their corresponding spindles. Meanwhile the lower nuclei of the chalazal region develop into a spireme in a more or less abortive manner as indicated in fig. 23. One of the megasporial nuclei is shown in polar view in fig. 24, and plate 30, figs. 4, 5 and 6, twelve chromosomes can be easily counted. In figs. 25*a* and *b*, the early stage of the eight nuclei embryo-sac is represented. The four nuclei towards the micropylar end and the two upper nuclei of the chalazal end are in the latest stage of telophase while the two daughter nuclei in the lower chalazal end are still in the early stage of telophase. The spindle fibers connecting the daughter nuclei are still in the complete form with the corresponding thickening in equatorial regions.

In figs. 26*a* and *b*, the eight nuclei are more or less separated from one another by thin membrane. One of the four micropylar nuclei and one of the four chalazal nuclei are proceeding towards the center of the embryo-sac to fuse with each other. The micropylar nucleus is distinctly smaller than the chalazal nucleus. One of the lower nuclei of the chalazal end is in the process of degeneration.

In fig. 27 the entire embryo-sac is represented in the lower magnification. Towards the micropylar end, the egg and the two synergids are somewhat distinctly differentiated. In the middle region, just below the large vacuole are shown the actual fusion of the polar nuclei and the three antipodal cells or nuclei at the extremity of



J. K. Santos

Figs. 25-27. 25 a. The micropylar end of the embryo-sac showing the four nuclei in a late telophase.  $\times 625$ . 25 b. The four chalazal nuclei in late metaphase.  $\times 625$ . 26 a. The micropylar end showing the differentiation of the four nuclei into egg and synergids.  $\times 625$ . 26 b. The chalazal end with the three antipodal cells and a large polar nucleus.  $\times 625$ . 27. A complete eight nucleate embryo-sac showing the egg, the synergid cells, the antipodal cells and the fusing polar nuclei.  $\times 450$ .

the embryo-sac. The lower nucleus is degenerating. A type of embryo-sac of this kind has been reported recently by the following investigators: Bambacioni (1) in *Fritillaria persica*, Bambacioni and Giombini (2) in *Tulipa Gesneriana*, Bambacioni-Mezzetti (3) in *Lilium candidum*, *L. bulbiferum*, and *Tulipa praecox*, and Cooper (4) in *Lilium Henryi*.

### Discussion

It will be seen from the foregoing that the megasporocyte of *Lilium philippinense* Baker is initiated by the enlargement of a subepidermal cell of the nucellus. This large subepidermal cell develops directly into the embryo-sac without any previous divisions. Such type of an embryo-sac formation is very similar to the embryo-sac development of different species of *Lilium* and to that of some other members of the *Liliaceae*, which have been reported by various earlier investigators.

The megasporocyte of *Lilium philippinense* passes into heterotypic and homoeotypic divisions in a normal way. The haploid number of the chromosomes is twelve and the diploid number is twenty-four. This diploid number agrees with the somatic number obtained by Satô (16) in *Lilium philippinense* var. *formosanum* Wilson.

The four resulting nuclei in the embryo-sac after the homoeotypic division are of the same size and contain the same number of chromosomes. Three of the four nuclei move towards the chalazal end and one to the micropylar end, and then divide simultaneously; the spindles of the three nuclei unite, forming a multipolar spindle. The chromosomes become attached to these spindles which ultimately develop into a single bipolar spindle. In this division, the chromosomes of the two unequal spindles divide somatically. As a result of this division, a four-nucleate embryo-sac is again formed. Two of the four resulting nuclei at the micropylar end are small and contain twelve chromosomes each, while the other two at the chalazal end are more voluminous and each contains thirty-six chromosomes.

Such difference in size and in number of chromosomes between the micropylar nuclei and chalazal nuclei during the development of the embryo-sac of various species of *Lilium* and of some other members of the *Liliaceae*, have been observed and reported by several earlier investigators. Among these may be cited Guignard (9, 10), Strasburger (17, 19), Dixon (7), Sargent (15), Mottier (12), Coulter (5), and Frisendahl (8). But it is quite surprising that no satisfactory explanation has been given until recently. Practically all of these investigators seem to believe that the cause of the said irregularity is either due to the difference in nutrition or to the splitting or segmentation of the chromosomes.

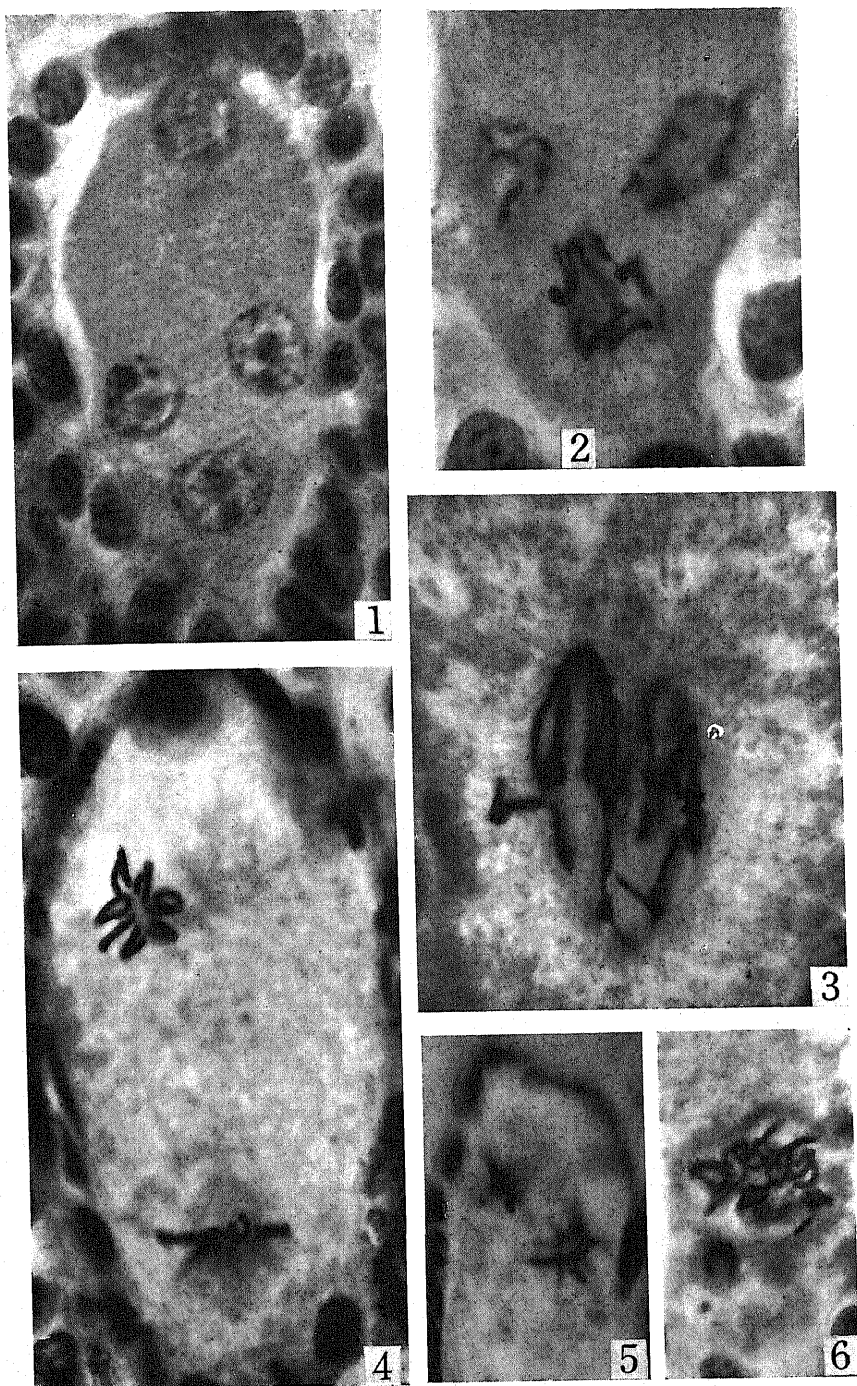
Recently Bambacioni (1) reported that in *Fritillaria persica* three of the four nuclei resulting from the homoeotypic division become closely arranged in the chalazal end, and one moves towards the micropylar end. All four nuclei divide simultaneously; the spindles of the three nuclei become united, forming a multipolar spindle,

which ultimately develops into a bipolar spindle. In view of this a four-nucleate embryo-sac is again formed in which the two daughter nuclei at the micropylar end are smaller and contain twelve chromosomes each and the two at the chalazal end are larger and contain thirty-six chromosomes each. Similar fusion was observed later by Bambacioni and Giombini (2) in *Tulipa Gesneriana* and by Bambacioni-Mezzetti (3) in *Lilium bulbiferum* and *Tulipa praecox*. Very recently Cooper (3) found the same fusion of three of the four nuclei, which had been formed as a result of the homoeotypic division in *Lilium Henryi*. The results obtained by the writer, therefore, in the present investigation of *Lilium philippinense* are fully in accord with the findings of Bambacioni and Cooper (4).

### Summary

1. The megasporocyte of *Lilium philippinense* is derived by the enlargement of subepidermal cell of the nucellus. The megasporocyte nucleus develops directly into an embryo-sac without any previous divisions.
2. The microsporocyte remains for a short time in the resting stage and immediately proceeds into heterotypic and homoeotypic divisions.
3. The haploid number of the chromosomes is twelve and the diploid number is twenty-four.
4. The four daughter nuclei resulting from the homoeotypic division are of the same size and contain the same number of chromosomes. Three of these four nuclei are closely arranged in the chalazal end, and the remaining one moves towards the micropylar end. Following this arrangement the four nuclei divide simultaneously. The spindles of the three nuclei become united forming a multipolar spindle which ultimately develop into a bipolar one.
5. Of the four resulting nuclei from this division, the two in the micropylar end have smaller dimension and contain twelve chromosomes each, while the two at the chalazal end are more voluminous and contain thirty-six chromosomes each.
6. The two pairs of nuclei resulting from the last division divide almost simultaneously forming an eight-nucleate embryo-sac. Two of the nuclei move towards the micropylar end and become synergids, one becomes into an egg, and the other moves towards the middle part of the embryo-sac and fuses with one of the chalazal nuclei, forming the polar nuclei. The three remaining chalazal nuclei move towards the extremity of the embryo-sac and become the antipodal nuclei.





Santos: Macrosporogenesis of *Lilium philippinense* Baker



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## Literature Cited

1. Bambacioni, V. Ricerche sulla ecologia e sulla embriologia di *Fritillaria persica* L. Ann. di Bot. vol. 18, 1930, 7-32.
2. — and Giombini, A. Sullo sviluppo del gametofito femminile in *Tulipa Gesnerians* L. Ibid. p. 373-386.
3. — and Mezzetti, V. Nuove ricerche sull'embriologia delle *Gigliacee*. Ibid. vol. 19, 1932, p. 365-382.
4. Cooper, D. C., Development of the embryo-sac of *Lilium Henryi*. Proc. Nat. Acad. Sci. vol. 20, No. 3, 1934, p. 163-166.
5. Coulter, J. M., Chamberlain Ch. J., Schaffner J. H., Contribution to the life history of *Lilium philadelphicum*. Bot. Gaz. vol. 23, 1897, p. 412-452.
6. Digby, L., On the archesporial and meiotic mitosis of *Osmunda*. Ann. Bot. vol. 33, 1919, p. 153-172.
7. Dixon, H. H. On the chromosomes of *Lilium longiflorum*. Proceedings of the Roy. Irish Acad., Ser. III Vol. 3, 1895 p. 707-719.
8. Frisendahl, A., Cytologische und entwicklungsgeschichtliche Studien an *Mycricaria germanica*. Kungl. Sv. Vet. Akad. Handl. Bd. 48, N. 7, 1912, p. 1-62.
9. Guignard, L., Recherches sur la structure et la division du noyau cellulaire chez des végétaux. Ann. des. Sc. Nat. Ser. 6, 1884, p. 5-59.
10. — Nouvelles recherches sur le noyau cellulaire. Ibid. 6, 1885, p. 310-372.
11. — Nouvelles études sur la fécondation. Ibid. VII. 14, 1891, p. 163-296.
12. Mottier, D. M., Über das Verhalten der Kerne bei der Entwicklung des Embryosacks und die Vorgänge bei der Befruchtung. Jahrb. f. wiss. Bot., Bd. 31, 1898, p. 125-158.
13. Overton, E., Beitrag zur Kenntniss der Entwicklung und Vereinigung der Geschlechtsproducte bei *Lilium martagon*. Zurich. 1891.
14. Santos, J. K. Determination of sex in *Elodea*. Bot. Gaz. vol. 77, 1924, p. 353-371.
15. Sargent, E. The formation of the sexual nuclei in *Lilium martagon*. Ann. of Bot., vol. 10, 1896, p. 107-108.
16. Satô, M., Chromosome studies in *Lilium* I. Bot. Mag. (Tokyo) vol. 46, 1932, 68-87.
17. Strasburger, E. Über den Theilungsvorgang der Zellkerne und das Verhältnis der Kerntheilung zur Zelltheilung, Bonn, 1882. p. 1-115.
18. — Neue Untersuchungen über den Befruchtungsvorgang bei den Phanerogamen. Jena. 1884.
19. — Chromosomenzahlen, Plasmastrukturen, Vererbungsträger und Reduktionsteilung. Jahrb. f. wiss. Bot., Bd. 45, 1908, p. 479-570.
20. Treub, M., et Mellink, J. F. A. Notices sur le développement du sac embryonnaire dans quelques Angiospermes. Archives neerl. d. Sc. exact. et. nat., vol. 15, 1880, p. 452-457.

## Explanation of Plate 30

*Lilium philippinense* Baker

- Fig. 1. An embryo-sac with four megasporial nuclei in the resting stage,  $\times 800$ .  
 Fig. 2. The chalazal end of the embryo-sac showing the three chalazal megasporial nuclei preparatory to the third division,  $\times 1250$ .  
 Fig. 3. The chromosomes and spindles of the three chalazal nuclei becoming united,  $\times 1250$ .  
 Fig. 4. A metaphase stage of the fourth division of the embryo-sac showing the polar view of one of the micropylar nuclei with twelve chromosomes and a portion of the side view of one of the chalazal nuclei,  $\times 800$ .  
 Fig. 5. A metaphase stage of the two nuclei at the micropylar end in the fourth division; the one in the polar view has twelve chromosomes,  $\times 666$ .  
 Fig. 6. An early stage of the metaphase of the upper chalazal nucleus in the fourth division,  $\times 666$ .

**Strukturelle Veränderungen im Gewebe der Stoffwechselzellen des  
„Fettkörpers“ von *Calliphora* während des Umbaues der Puppe**  
Vorläufige Mitteilung

Von

**R. J. H. Teunissen**

Aus der Abt. f. exper. Morphologie des Zool. Labor., Universität, Utrecht  
(Leiter: G. C. Hirsch)

Während des Puppenstadiums macht *Calliphora* weitgehende Veränderungen im Aufbau des Gewebes durch: nur das Nervensystem, eine Gruppe von Speicheldrüsen, die Geschlechtsorgane und schließlich einige Tracheen werden vom larvalen Körper in den Imago übernommen. Alle anderen Gewebe werden vollständig abgebrochen und auf neue aufgebaut. Bei dem Ab- und Aufbau spielt der sogen. „Fettkörper“ eine gewisse Rolle. Das Laboratorium für experimentelle Morphologie des zoologischen Institutes der Universität Utrecht hat sich in dem letzten Jahre mit diesen Veränderungen und ihrer Determination befaßt und es werden in der nächsten Zeit einige Publikationen hierüber erscheinen. Uns beschäftigt hier zunächst die Frage nach der Tätigkeit des „Fettkörpers“ während des Puppenzustandes.

Die Frage ist darum für uns besonders interessant geworden, weil es uns sehr wahrscheinlich geworden ist, daß die Zellen des „Fettkörpers“ während des Abbruches der anderen Gewebeteile die freiwerdenden Stoffe aufnehmen, intraplasmatisch verarbeiten und dann erneut den sich bildenden Geweben zur Verfügung stellen. Die Zellen des „Fettkörpers“ haben also eine Art Fabrikationstätigkeit neuer Stoffe, die sie aus alten Stoffen aufbauen: sie sind eine „Resteverwertungs-fabrik“. Welchen Namen müssen wir ihnen dann beilegen? Es ist nicht sicher, ob sie wirklich phagozytieren können; wir haben vielmehr den Eindruck gewonnen, alsob sie nur chemisch tiefer abgebaute Stoffe in sich aufnehmen und haben deswegen davon abgesehen, diese Zellen Phagozyten zu nennen. Wir wollen ihnen aber den Namen „Stoffwechselzellen“ beilegen, welcher Name am deutlichsten ausdrückt, daß es sich hier um Zellen handelt, denen die eigentliche Aufgabe zufällt, die alten Gewebereste chemisch so wieder aufzubauen, daß sie dann später dem neuen Gewebe zu Gute kommen können.

Wir gehen in dieser vorläufigen Mitteilung noch nicht auf die bisherige Literatur ein, sondern teilen nur in Kürze einen Teil unserer

bisherigen Ergebnisse mit. Wir beschränken uns dabei auf die Beantwortung der folgenden Fragen:

1. Welches ist der Verband der Stoffwechselzellen im Fettkörper? Arbeiten die Zellen als einzelne Zellen oder als Sekundäre Syncytia? (unter Sekundären Syncytia verstehen wir das Zusammenfließen von Zellen zu einer größeren Plasmamasse mit zahlreichen Kernen) G. C. HIRSCH 1929.

2. Welche Strukturveränderungen sind mit zunächst einfacher mikroskopischer Technik in den Zellen und ihren Kernen während der Tätigkeit der Stoffwechselzellen zu beobachten? (Wir haben also in dieser Abhandlung vorläufig noch außer Acht gelassen, welche Stoffe in die Zellen eindringen, hier verarbeitet und wieder abgegeben werden).

### Technik

Die für diese kleine Arbeit zunächst angewandte Technik war denkbar einfach: vorallem Ausstrichpräparate der verschiedensten Stadia der Puppenentwicklung in zahlreichen Stufen; diese wurden fixiert in BOUIN und gefärbt in Haemalaun und Eosin. Außerdem haben wir zahlreiche Schnitte gemacht, welche aber bisher noch nicht in dieser Arbeit verarbeitet worden sind; sie werden zusammen mit den Ergebnissen der Vitalfärbung und der chemischen Untersuchungen demnächst publiziert werden.

### Einige Beobachtungen über die Stoffwechselzellen während des Larvenstadiums

Im Larvenstadium sind die Organe des Körpers noch intakt. Der „Fettkörper“ ist bereits gebildet, ruht aber in seiner eigentlichen Stoffwechseltätigkeit (Abb. 1). Die Stoffwechselzellen sind ziemlich klein. Sie liegen in einem deutlichen Gewebeverbande und sind durch eine sehr dünne Schicht umgeben; die sogenannte Tunica, welche alle Zellen zu einem einheitlichen Gewebe verbindet. Abb. 10 zeigt, daß sich in dem Plasma zahlreiche kleine Fettvakuolen befinden, dagegen relativ wenige Granula. Während dieser Periode bilden die Stoffwechselzellen schon Reservestoffe, von denen wohl das Fett den Hauptstoff bildet; außerdem sind Glykogen (Abb. 16), Fettsäuren und Eiweiße in Form von Granula ebenfalls vorhanden, aber in viel geringerem Grade. Die Kerne (Abb. 17) sind verhältnismäßig klein; ihre Oberfläche ist glatt, ihre Chromatinmasse ist gleichmäßig, vorallem am Rande des Kernes verteilt; Nukleolen sind ziemlich selten. Dies ist also der Zustand, in welchem die Stoffwechselzellen einige Reservestoffe, vorallem Fett, in sich aufgestapelt haben; und dieses scheint uns die Hauptmenge der Reservestoffe zu

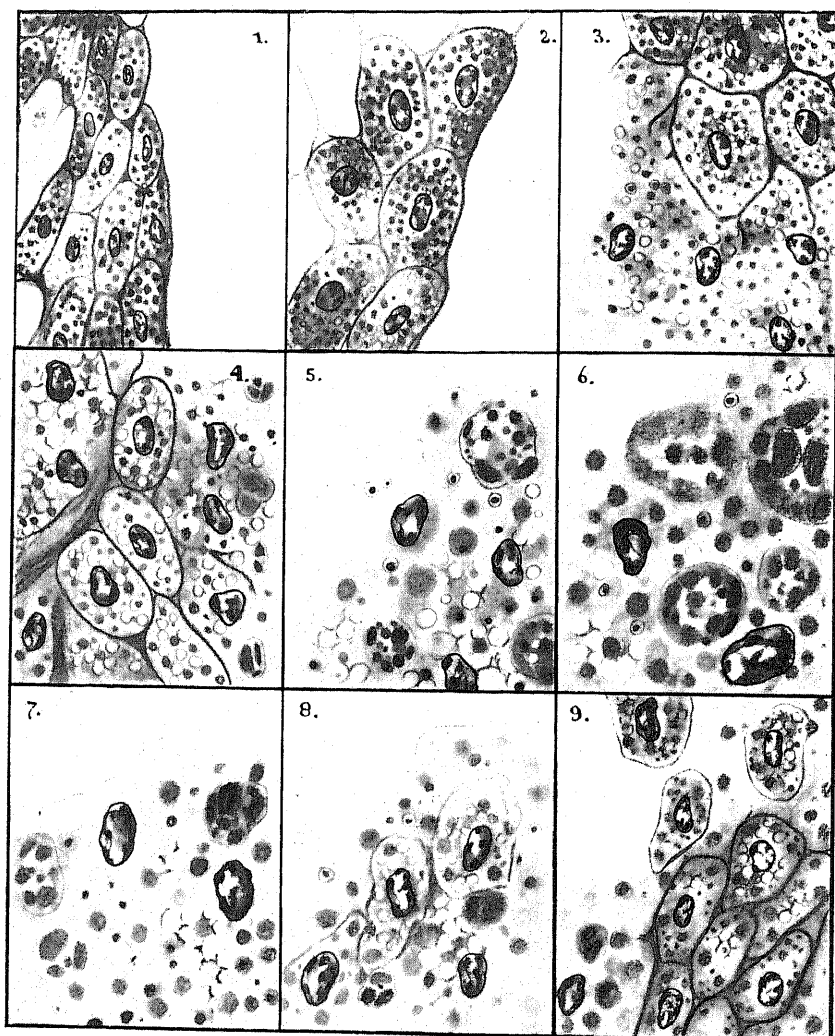


Abb. 1-9: Veränderungen im Verlande der Stoffwechselzellen des Fettgewebes. 1: Ein Fettkörper am Ende der larvalen Periode. Das Gewebe ist umgeben von einer dünnen Tunica. 2: Ein frühes Puppenstadium, etwa eine halbe Stunde nach dem Unbeweglichwerden der Larve; die Stoffwechselzellen besitzen jetzt bereits mehr Granula; ihre Kerne haben an Größe etwas zugenommen. 3: 6 Stunden nach Verpuppung. An vielen Stellen wird die Zellmembran gesprengt und ein Sekundäres Synzytium bildet sich: die Kerne liegen unregelmäßig verteilt zwischen dem Sekundären synzytialen Plasma, den Granula und den Vakuolen. 4: 24 Stunden nach Verpuppung: nur noch wenige intakte Zellen sind übrig, die in kleinen Gruppen bei einander liegen; der größte Teil des Gewebes ist Sekundäres Synzytium geworden; rechts liegt der Rest einer Zellwand. 5: 48 Stunden nach Verpuppung. Die Kerne des Sekundären Synzytiums sind größer geworden. Die Granula im Synzytium haben an Größe stark zugenommen; es erscheinen die ersten Zusammengesetzten Granula. Zustand stärksten Stoffwechsels im Synzytium. 6: 72 Stunden nach Verpuppung. Die Anzahl der Zusammengesetzten Granula und ihre Größe hat zugenommen; die

sein, welche die Larve erwirbt, um sie im Puppenstadium zu gebrauchen.

Ist das Ende der larvalen Periode erreicht, so sind die Stoffwechselzellen größer geworden als im Anfange dieser Periode. Ebenso nahmen nun die Nukleolen an Anzahl zu, und schließlich wird die Oberfläche des Kernes unregelmäßiger und die Chromatinmasse wird mehr aufgehäuft (Abb. 18).

### Die Umsetzungen während des Puppenstadiums

Die Abb. 2 zeigt die Stoffwechselzellen eine halbe Stunde nach dem Unbeweglichwerden der Larve: der Zellverband ist derselbe geblieben. Aber die Stoffwechselzellen selbst besitzen zahlreichere Granula als im Larvenstadium. Schon in den ersten 3–4 Stunden nimmt bereits die Anzahl der Granula stark zu (Abb. 11), während relativ wenige Fettvakuolen zu sehen sind (Abb. 15). Es treten 2 verschiedene Formen von Granula auf: solche, die sich nach Färbung mit Haemalaun-Eosin stark rot färben und solche, welche lila gefärbt sind; sie sind in der Zeichnung durch verschieden starke Schattierung angegeben. Es sind dies diejenigen Granula, welche sich formen auf Grund des Eintretens von Rohstoffen in das Innere der Zellen während des Puppenstadiums. Abb. 12, welche etwa 4 Stunden nach dem Beginn des Puppenstadiums gezeichnet wurde, zeigt, daß die Größe der Granula zugenommen hat. Es wird in diesen ersten 6 Stunden durch diese Stoffwechselzellen außergewöhnlich Viel in Form von Granula aufgestapelt.

Hierdurch—so möchten wir annehmen—entsteht wohl ein stärkerer Innendruck in den Zellen. Vielleicht ist es dadurch zu erklären, daß die Zellwände etwa 6 Stunden nach Beginn des Puppenstadiums gesprengt werden: Abb. 3 und 13. Hierdurch ergießt sich der Inhalt der Zellen in eine allgemeine Plasmamasse hinein, die durch ein Zusammenfließen der Zellen entsteht.

Diese auffallende syncytiale Plasmamasse entsteht an verschiedenen Punkten des Fettkörpers zu gleicher Zeit. Die Folge dieser sekundären Syncytiumbildung, der sich allmählich

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Größe vieler solcher Zusammengesetzter Granula übertrifft die Größe der ursprünglichen Zelle. 7: 144 Stunden nach Verpuppung. Die Anzahl der Zusammengesetzten Granula hat schon etwas abgenommen. 8: 216 Stunden nach Verpuppung: mit einem Kern (als Organisationszentrum?) in der Mitte treten die ersten Zellwände auf. Die großen Zusammengesetzten Granula sind fast ganz verschwunden, d.h. ihre Stoffe sind an das Restitutionsgewebe abgegeben. 9: Imago 2 Stunden nach dem Ausschlüpfen. Reste des Syncytiums sind nur spärlich links unten zu sehen; die größte Masse des Plasmas ist wieder zellig organisiert: d.h. die meisten Zellen haben sich wieder zu einem Zellverbände, d.h. einem Gewebe zusammengefunden;

nur einige Zellen bleiben vorläufig noch isoliert.

der ganze Fettkörper anschließt, ist, daß in einer gemeinschaftlichen Plasmamasse Zellkerne, Granula, Fettvakuolen und Plasmateile durcheinander schwimmen. Und diese syncytiale Masse verteilt sich über zahlreiche Plätze der gesamten Puppe. Die Kerne bleiben als

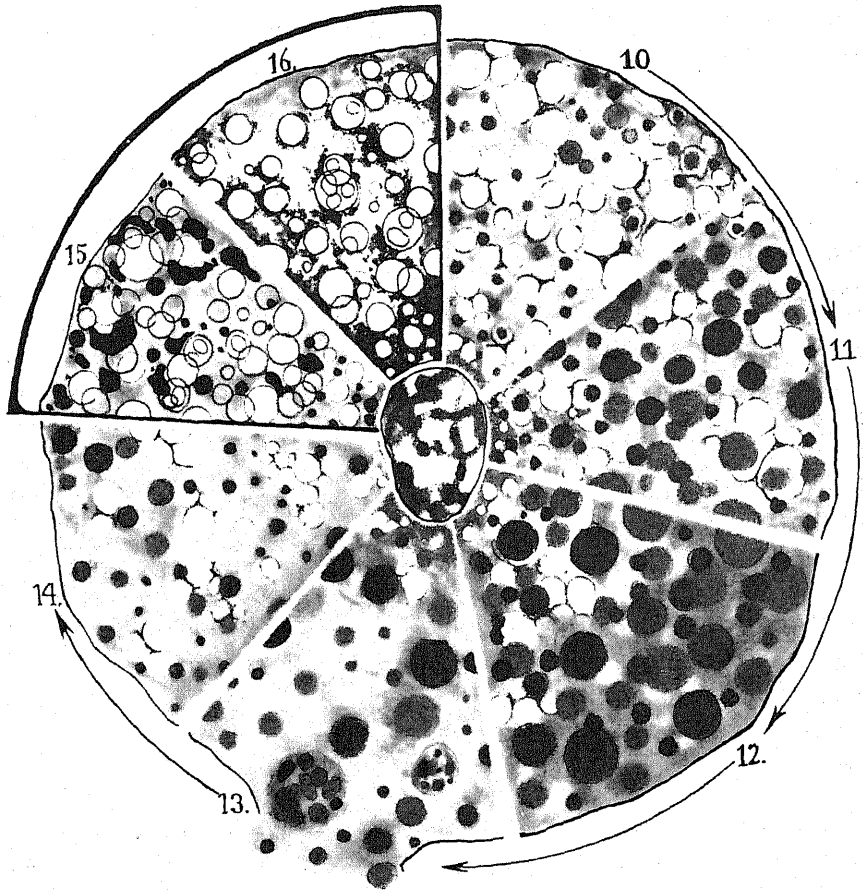


Abb. 10-16: Strukturveränderungen im Plasma der Stoffwechselzellen. 10: Am Ende des larvalen Zustandes: zahlreiche Fettvakuolen (weiß) und etwas weniger Eiweiß-Granula verschiedener Konstitution. 11: Beginn des Puppenstadiums: die Anzahl der Granula hat gegenüber den Fettvakuolen bedeutend zugenommen. Das einzelne Granulum ist gewachsen. Die beiden Arten der Granula (mit Eosin oder Haemalaun färbbar) sind hier unterschieden durch die Schattierung in der Zeichnung. 12: Etwa 4 Stunden nach der Verpuppung: das Wachstum der Granula hat noch stark zugenommen. 13: Die Zellgrenze wird zersprengt; der Zellinhalt ergießt sich in ein gemeinschaftliches Sekundäres Synzytium. Es bilden sich die ersten Zusammengesetzten Granula. 14: Ende des Puppenstadiums. Stadiums der erneuten Zellbildung. Das Stadium ähnelt der Abb. 10, enthält aber wesentlich weniger Fett. Die Zusammengesetzten Granula sind ganz verschwunden. 15: Etwa 4 Stunden nach der Verpuppung. Das Plasma ist mit Osmiumsäure behandelt. Schwarz: das Fett; weiße oder graue Zirkel: die Granula. 16: Ältere Larve, etwa 2 Stunden vor der Verpuppung. Darstellung des Glykogens nach BEST, hier schwarz gefärbt; Granula weiß.



solche erhalten (Abb. 19 und 20); sie nehmen aber an Größe zu und ihre Oberfläche wird unregelmäßiger. Vorläufige Messungen haben ergeben, daß die Zunahme der Kerngröße beträchtlich ist; wir kommen später auf diese Frage zurück.

24 Stunden nach der Verpuppung (Abb. 4) sieht man von dem Fettgewebe nur noch einige wenige Zellgruppen. Es herrscht im Bilde das Syncytium vor.

48 Stunden nach der Verpuppung (Abb. 5) finden sich nur noch ganz wenige intakte Stoffwechselzellen. Von nun ab werden die synthetischen Stoffwechselprozesse auch mikroskopisch uns deutlich gemacht durch das Auftreten von „Zusammengesetzten Granula“. Diese sind schon im Stadium der Abb. 13 zum ersten Male deutlich (2 Stück in der Zeichnung); es werden ihrer von nun ab je länger je mehr; besonders Abb. 6 und 7 zeigt ihre zunehmende Häufigkeit. Diese Granula können größer werden als früher die ganze Zelle war! Wir haben Argumente für die Annahme, daß es sich hier um die Bildung kleiner „Stoffwechsellaboratoria“ handelt, in welchen bestimmte chemische Aufbauprozesse durch eine Abschlußmembran sich abschließen gegen die übrige Plasmamasse. Die Prozesse, die in diesen Granula stattfinden, scheinen vorallem Eiweiß aufzubauen.

Etwa 70 Stunden nach Verpuppung (Abb. 6) ist der größte Teil des Gewebes innerhalb der Puppe ein dünnflüssiger Brei geworden; die larvalen Muskeln sind abgebrochen; die Darmwandzellen sind auch auf mehreren Stellen schon auseinander gefallen. Die ersten Restitutionen zur Bildung des imaginalen Gewebes haben eingesetzt. —Jetzt hat die Anzahl und Größe der Zusammengesetzten Granula wohl den Höhepunkt erreicht.

Abb. 8 zeigt die Verhältnisse der Stoffwechselzellen 216 Stunden nach der Verpuppung. Es ergibt sich, daß in dieser Zeit die Masse der in den Stoffwechselzellen umgesetzten Granula und Fetten schon wieder beträchtlich abgenommen hat. Diese Stoffe sind inzwischen an das Restitutionsgewebe zum Aufbau des imaginalen Körpers abgegeben worden.

Das Protoplasma (Abb. 14) ähnelt in seinem Zustande jetzt etwa wieder dem Plasma der Stoffwechselzellen am Ende der larvalen Periode. Doch ergibt sich ein Unterschied: die Anzahl der Fettvakuolen ist jetzt wesentlich geringer. Langsam verschwinden auch die Zusammengesetzten Eiweißgranula, je nachdem ihre Stoffe von den Restitutionsgeweben aufgenommen worden sind.

An den Kernen, welche noch frei im Syncytiumplasma liegen, zeigen sich (Abb. 22) eigentümliche Zusammenballungen von Chromatin; von einem Netzwerke des Chromatins kann jetzt nicht mehr

gesprochen werden. Eigentümlicherweise finden sich solche Körper auch außerhalb der Kerne im syncytialen Protoplasma. Wir können uns über die Bedeutung dieser Erscheinungen vorläufig noch nicht näher aussprechen, da die Analyse noch nicht abgeschlossen ist.



Abb. 17-24 zeigen den Wechsel der Kernform während der Arbeit der Stoffwechselzellen. 17: Kern einer Stoffwechselzelle der Larve, einige Tage vor dem Beginn des Puppenstadiums. 18: Kern aus der Zeit des Endes des Larvenstadiums. 19-21: frühes Puppenstadium: starke Größenzunahme der Kerne, kompakteres Chromatin vieler Nukleolen. 22: Auftreten von Chromatin-Zusammenballungen noch unbekannter Bedeutung. 23: Ende des Puppenstadiums: Zurückgehen der Zusammenballungen des Chromatins und geringere Färbbarkeit der Chromatinmasse. 24: Stadium des hungrigen Imago; erneute Zellbildung; Struktur etwa gleich Abb. 17.

Wir halten es aber für möglich, daß es sich hier um Nukleolen handelt, welche, aus dem Kerne austretend, vielleicht eine Stoffwechselbedeutung im Protoplasma selbst haben; doch gibt es auch noch andere Möglichkeiten.

Bei einer Puppe von etwa 9 Tagen beginnen nun eigentümlicherweise in den Resten des Sekundären Syncytiums langsam wieder Zellwände aufzutreten (Abb. 8): es entstehen im Plasma nesterweise Wände, welche eine bestimmte Protoplasmanasse und einen Kern umschließen. Es ist deutlich, daß der Kern der Mittelpunkt der Zellbildung ist—und wir dürfen wohl daraus die Hypothese ableiten, daß die Kräfte, welche die Zellbildung

zuwege bringen, ihre Determination im Kerne haben. Damit würde das alte Wort von SCHWANN (1839) in anderem Sinne wiederaufleben: „Wenn der Kern eine gewisse Entwicklungsstufe erreicht, so bildet sich um ihn die Zelle“; SCHWANN dachte an freie Zell- und Kernbildung; wir suchen nur nach den determinierenden Faktoren der Wandbildung.

Das Protoplasma ist in Übereinstimmung mit der Abb. 14 und die Kerne mit der Abb. 22: die Struktur der Kerne wird wieder weniger deutlich, das Chromatin nimmt weniger stark Farbstoffe auf und die Kernmembran wird wieder regelmäßiger. Damit ist die Tätigkeit der Stoffwechselzellen während des Puppenstadiums beendet.

### **Die Stoffwechselzellen während des ersten imaginalen Lebens**

Wenn der Imago ausgeschlüpft ist, so sind nur noch wenige syncytiale Bildungen vorhanden: d.h. die Zellen des reduzierten „Fettkörpers“ haben sich wiederum gebildet; sie sind jetzt aber wesentlich kleiner als im Anfang des Puppenstadiums. Abb. 9 läßt erkennen, daß es sich fast überall Zellen gebildet haben. Sie enthalten etwa denselben Zellinhalt, wie die Abb. 14 wiedergibt. Abb. 24 zeigt die durchschnittliche Kernform, welche einige Übereinstimmung besitzt mit den Kernen der Stoffwechselzellen am Ende der larvalen Periode (Abb. 17).

Die weiteren Untersuchungen werden die Fragen erörtern, was, während der Synzytiumbildung der Stoffwechselzellen, in ihnen vor sich geht und welches das Schicksal der angeführten und abgeführten Stoffe ist.

### **Zusammenfassung**

Die Arbeit zeigt als vorläufige Mitteilung die strukturellen Veränderungen in den Stoffwechselzellen des Fettkörpers. Diese Zellen dienen dazu, die große Masse des sich auflösenden Gewebes der Puppe in sich aufzunehmen und intraplasmatisch zu verarbeiten. Nach der Verarbeitung werden die Stoffe den Restitutionsgeweben der Puppe wiederum zur Verfügung gestellt. Hierbei zeigen sich folgende gröbere strukturelle Veränderungen an den Stoffwechselzellen:

Abb. 1–9 zeigen den Wechsel des strukturellen Zusammenhanges der Stoffwechselzellen: zuerst liegen die Zellen im Gewebeverbande, von einer Tunica gemeinsam umgeben. Dann werden die Zellgrenzen gesprengt, und die Kerne kommen frei in einer Plasmamasse zu liegen. In dieser Sekundären Syncytialen Plasmamasse werden große Mengen von zwei verschiedenen Granula gebildet: Aufbau neuer Eiweißstoffe. Es bilden sich auch sehr große Zusammen-

gesetzte Granula, die als kleine Stoffwechselfabriken gedeutet werden; diese können größer werden als die ursprüngliche Zelle. Diese aufgebauten Stoffe werden an das Restitutionsgewebe später abgegeben. Schließlich geht das Sekundäre Syncytium wieder in einen Zellverband über, indem sich um jeden Kern und etwas Protoplasma eine Zellmembran bildet; es wird angenommen, daß die Determination der Zelle vom Zellkerne ausgeht.

Die Abb. 10–14 zeigen die Veränderungen im Protoplasma der Stoffwechselzellen: zuerst vor allem Fett, dann zwei verschiedene Arten von immermehr wachsenden Granula; schließlich werden die Zellgrenzen gesprengt. Die Stoffwechselzellen gehen ein in den Imago, mit einem Plasma entsprechend Abb. 14.

Abb. 17–24 lassen die Kernveränderungen der Stoffwechselzellen erkennen und zeigen: die Kerngröße wechselt und erreicht etwa in der Mitte des Puppenstadiums den größten Inhalt. Gegen das Ende der Hauptstoffwechselprozesse treten in den Kernen große Nukleolen auf, welche auch teilweise im Plasma, außerhalb des Kernes, gefunden werden; ihre Bedeutung ist noch unsicher.

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## Studies on the Chromosome Numbers in Higher Plants, with Special Reference to Cytokinesis, II

By

T. Sugiura

Osaka Higher School, Osaka

The studies on the numbers of chromosomes in plants have been carried out to an increasingly greater extent and they have indeed contributed to the progress in experimental genetical studies, though the work that has been done is nevertheless still far from being sufficient, especially among families rather than in a genus. The present work is a continuation of the studies which have been in progress since 1926.

### Material and methods

Materials used for this study were taken from 27 species, belonging to 18 genera, raised from seeds which were imported from Europe. The reagent used for fixing the materials was the Farmer's fluid which was modified by the writer and used since 1920, because he believes that chromosomes should be fixed under conditions as nearly uniform as possible in order to compare their size and form in different plants examined.

### The mode of cell division

The plants described below all show the furrowing process in the mode of partition wall formation of the pollen mother cells (cf. Sugiura 1936b).

### Numbers of the chromosomes

*Chrysanthemum rupestre* (Compositae) (Fig. 1).

It has 9 meiotic chromosomes as other species in this genus. They are V-shaped at the first anaphase.

*Acicarpa tribuloides* (Calyceraceae) (Fig. 2).

This family has been quite unknown in karyological studies except Dahlgren's (1915). The pollen mother cells are smaller than those of the above mentioned *Chrysanthemum* and especially the filar shaped meiotic chromosomes much smaller than the latter. The meiotic chromosomes are 8 in number, while Dahlgren thought there were about 8.

*Stylidium adnatum* (Stylidiaceae) (Fig. 3).

There has been no chromosome count in this family up to the present time. The meiotic chromosomes, being spherical at the

second metaphase, are 15 in number. The pollen mother cells are far larger than those of *Acicarpa* in spite of the fact that *S. adnatum* is of small size in our green house.

*Globularia cordifolia* (Globulariaceae) (Fig. 4).

This family has been little studied in chromosome counts. The meiotic chromosomes are spherical and 10 in number.

*Maurandia purpurea* (Scrophulariaceae) (Fig. 5).

The meiotic chromosome number is 12, 4 of which are in the centre, the remaining 8 making a ring.

*M. scandens* (Fig. 6).

The chromosome number in the somatic cells, according to Heitz, is 24, but the writer counted ten meiotic chromosomes, one of which is situated at the centre in the first meiotic metaphase.

*Browallia grandiflora* (Solanaceae) (Fig. 7), *B. demissa* (Fig. 8), *B. viscosa* (Fig. 9).

Formerly the writer studied some plants in *Browallia*, each of which had all 11 meiotic chromosomes except *B. speciosa* which had 22 meiotic chromosomes. He now also counted 11 meiotic chromosomes in the above mentioned species.

*Salvia dumetorum* (Lamiaceae) (Fig. 10).

The meiotic chromosome numbers hitherto studied in the genus *Salvia* were 9, 10 and 11, according to our investigation. We find the number 7 in the present species. According to Scheel (1930), the same number was also found in *S. pseudosilvestris* and *S. recognita*. But in recent years the same number was also found in *S. Candelabrum*, *grandiflora*, *nemorosa*, *officinalis* and *scabiosaefolia*.

Thus it seems that *Salvia* belongs to Tischler's *Carex* type. The meiotic numbers hitherto found in this genus are 6, 7, 8, 9, 10, 11, 12, 13, 16, 18, 20, 22 and 32.

*Heuchera sanguinea* (Saxifragaceae) (Fig. 11).

Formerly Schoennagel (1930) counted 7 meiotic chromosomes in this plant which has been confirmed by the present count. Quite recently Skovsted (1934) found 3 fragmental chromosomes in addition to the normal 7 meiotic chromosomes in a form of the present plant, but there were found no such chromosome fragments in our material.

*Cleome spinosa* (Capparidaceae) (Fig. 13).

Considering Taylor's, Tischler's, Ufer's and the writer's results the genus *Cleome* seems to belong to Tischler's *Carex* type. The meiotic number of chromosomes is 12, but Ufer counted 10.

*Cleome Candelabrum*  $n = 16$  (Fig. 12).

Meiotic chromosomes are spherical and smaller than those of *spinosa*.

*Dactylaema micrantha*  $n = 16$  (Fig. 14).

The genus *Dactylaema* has not yet been studied cytologically.

Table 1. Number and size ( $\mu$ ) of chromosomes in plants

Plants investigated	Fig.	Chromosome Number (n)	First metaphase ( $\mu$ )	First anaphase ( $\mu$ )	Second metaphase ( $\mu$ )	Second anaphase ( $\mu$ )
<i>Chrysanthemum rupestre</i>	1	9			$1.75 \times 0.75$	
<i>Acicarpa tribuloides</i>	2	8			$0.63 \times 0.35$	
<i>Stylidium adnatum</i>	3	15			1.00	
<i>Globularia cordifolia</i>	4	10	0.75			
<i>Maurandia purpurea</i>	5	12		0.62		
<i>M. scandens</i>	6	10	$0.75 \times 0.55$			
<i>Browallia grandiflora</i>	7	11		$2.0 \times 1.0$		
<i>B. demissa</i>	8	11		$1.5 \times 1.0$		
<i>B. viscosa</i>	9	11	$1.5 \times 1.0$			
<i>Salvia dumetorum</i>	10	7			0.75	
<i>Heuchera sanguinea</i>	11	7			$1 \times 0.75$	
<i>Cleome Candelabrum</i>	12	16		0.75		
<i>C. spinosa</i>	13	12				
<i>Dactylaema micrantha</i>	14	16		0.5		
<i>Cheridonium</i>		6	1.00			
<i>Franchetianum</i>	15					
<i>Glaucium corniculatum</i>	16	6				0.75
<i>Papaver Argemone</i>	17	21	1.0			
<i>P. dubium</i>	18	21	1.125			
<i>P. pinnatifidum</i>	19	14	1.13			
<i>P. commutatum</i>	20	7	1.25			
<i>P. alpinum</i>	21	7	1.3			
<i>P. oreophilum</i>	22	7	$1.13; 0.62$			
<i>Lychnis lagascae</i>	23	12			0.75	
<i>Portulaca pusilla</i>	24	9			$1.25 \times 0.7$	
<i>Calandrinia Menziesii</i>	25	24			0.5	
<i>C. procumbens</i>						
<i>Mesembryanthemum criniflorum</i>	26	9				
<i>Thelygonum japonicum</i>	27	11		1.25		

*Glaucium corniculatum* (Papaveraceae) (Fig. 16).

The meiotic number of chromosomes is 6 as in *G. Serpieri*.

*Papaver Argemone* ( $n = 21$ ) (Fig. 17), *P. dubium* ( $n = 21$ )

(Fig. 18), *P. pinnatifidum* ( $n = 14$ ) (Fig. 19), *P. com-*

*mutatum* ( $n = 7$ ) (Fig. 20), *P. alpinum* ( $n = 7$ ) (Fig. 21),

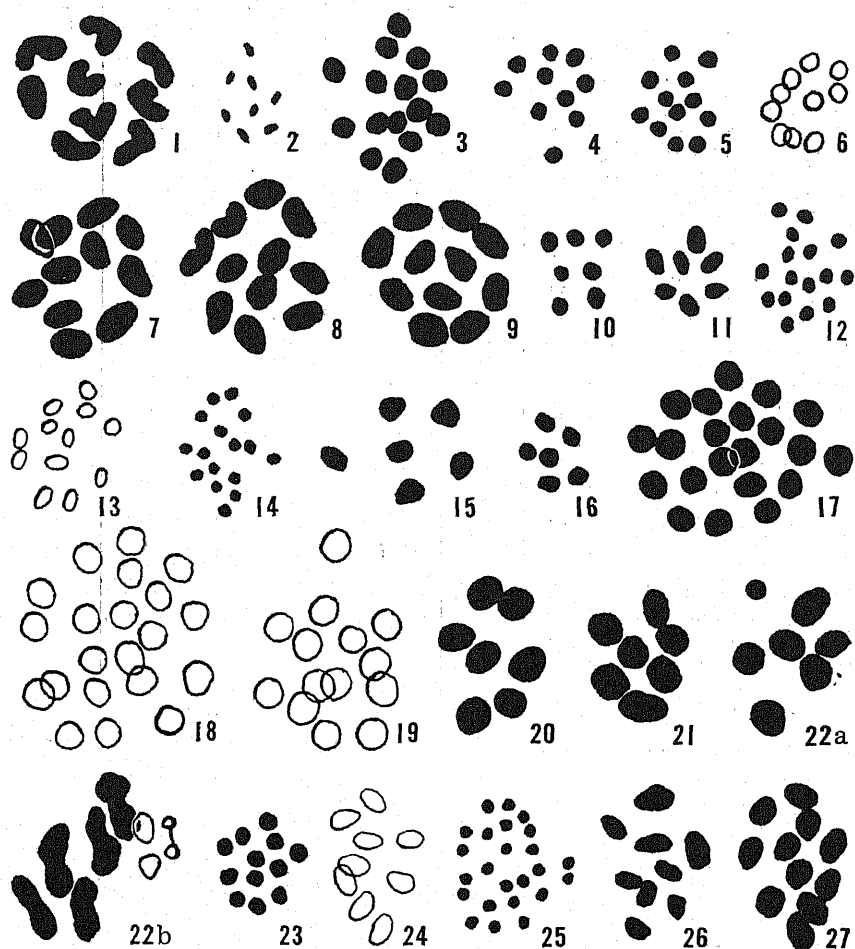
*P. oreophilum* ( $n = 7$ ) (Fig. 22).

Since Tahara's studies on the genus *Papaver*, the study of the chromosome count has become more and more active recently. Ljungdahl (1922), Yasui (1921), Winge (1925) were the chief contributors. The writer, having already counted the chromosome numbers in several species in this genus, has studied anew other species mentioned above.

In *Papaver* it seems that there are no such significant differences in chromosome sizes among diplont, tetraplont, hexaplont, etc. as far



as the present study is concerned. Those species having 7 meiotic chromosomes are *P. alpinum*, *commutatum* and *oreophilum*, among which the last species has 6 normal chromosomes and one very small one, as shown in Fig. 22. The meiotic number of chromosomes in *P. alpinum* and *dubium* were found by Ljungdahl (1922) to be 7 and 14, but the writer's count in *dubium* shows 21. *P. commutatum* was also found by Philip to have the same number 7.



Figs. 1-27.  $\times 3300$ . 1, *Chrysanthemum rupestre*. IA. 2, *Acicarpa tribuloides*. IIM. 3, *Stylidium adnatum*. IIM. 4, *Globularia cordifolia*. IM. 5, *Maurandia purpurea*. IA. 6, *M. scandens*. IM. 7, *Browallia grandiflora*. IA. 8, *B. demissa*. IA. 9, *B. viscosa*. IA. 10, *Salvia dumetorum*. IIM. 11, *Heuchera sanguinea*. IIM. 12, *Cleome Candelabrum*. IA. 13, *C. spinosa*. IM. 14, *Dactylaema micrantha*. IIM. 15, *Cheridonium Franchetianum*. IM. 16, *Glaucium corniculatum*. IIA. 17, *Papaver Argemone*. IM. 18, *P. dubium*. IM. 19, *P. pinnatifidum*. IM. 20, *P. commutatum*. IM. 21, *P. alpinum*. IM. 22, *P. oreophilum*. a. IM. b. IM. (side view). 23, *Lychnis lagascae*. IIM. 24, *Portulaca pusilla*. IIM. 25, *Calandrinia Menziesii*. IIM. 26, *Mesembryanthemum criniflorum*. IIM. 27, *Thelygonum japonicum*. IA.

*Lychnis lagascae* (Caryophyllaceae) (Fig. 21).

The genus *Lychnis* was previously studied by Blackburn (1928-29) and found to have 12 meiotic chromosomes. This species has also 12 meiotic chromosomes.

*Portulaca pusilla* (Portulacaceae) (Fig. 24).

Since Tjebbes counted 9 meiotic chromosomes in *P. grandiflora*, Hagerup, Okura and Blackburn also found the same number in the above named plant. The size of the pollen mother cells is rather larger in comparison with the external appearances of anthers. The furrowing process commences even in the second metaphase.

*Calandrinia Menziesii* (Fig. 25), *C. procumbens*.

The meiotic number of chromosomes in *Calandrinia* was first counted by the writer. Previously three species were studied and their meiotic chromosomes were found to be 8, 19, 12 and 24 respectively. Thus it seems that this genus is polyphylogenetic from the karyological point of view.

*Mesembryanthemum criniflorum* (Aizoaceae) (Fig. 26).

The writer has already studied some plants in *Mesembryanthemum* and counted 9 meiotic chromosomes in them. The same number is also found in this species.

*Thelygonum japonicum* (Thelygonaceae) (Fig. 27).

Thelygonaceae has only the genus *Thelygonum*, in which only 3 species are found, namely *Th. Cynocrambe*, *Th. macranthum* and *Th. japonicum*. The former two are annual while the latter is perennial. It is very interesting to note that Schneider (1913) found the meiotic number of chromosomes in *Th. Cynocrambe* to be 10, while the writer counted 11 in *Th. japonicum*.

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#### Literature

- Skovsted, A. 1934. Cytological Studies in the Tribe Saxifragaceae. Dansk Bot. Arkiv. 8: 30.  
Sugiura, T. 1936 a. A list of chromosome numbers in angiospermous plants. II. Proc. Imp. Acad. (Tokyo) 12: 144-146.  
— 1936 b. Studies on the chromosome numbers in higher plants, with special reference to cytokinesis, I. Cytologia 7: 544-595.  
Tischler, G. 1935/36. Pflanzliche Chromosomen-Zahlen. Nachtrag Nr. 2, Teil I. Tab. Biol. Period. 5/6.

## Untersuchungen über die Geschlechtszellen von *Spirogonium sticticum* Kutz.

Von

**Seikan Kusunoki**

Himeji-Kôtôgakkô, Himeji

(Mit 1 Tafel und 1 Textabbildung)

### Einleitung

Der Bau der vegetativen Zellen ist bei den Gattungen *Spirogyra* und *Sirogonium* sehr ähnlich. Deshalb sind viele Autoren geneigt, *Spirogyra* und *Sirogonium* als nur einer Gattung angehörig zu betrachten. Jedoch bestehen bei beiden deutliche Unterschiede in der Art der Kopulation. Die Frage, ob *Sirogonium* gemischt- oder getrenntgeschlechtlich ist, konnte bis heute nicht sicher entschieden werden. H. KNIEP<sup>1)</sup> schreibt:

„Darüber, ob *Sirogonium* getrenntgeschlechtlich oder gemischtgeschlechtlich ist, liegen einander widersprechende Angaben vor. DE BARY (1858) behauptet mit großer Bestimmtheit, daß sowohl Teile des gleichen Fadens, wie auch getrennte Fäden miteinander kopulieren können.

Demgegenüber gibt GAY (1884) an, daß nur Zellen verschiedener Fäden kopulieren.

Es bleibt zu prüfen, ob bei *Sirogonium sticticum* (Engl. bot.) WILLE, worauf sich beide Angaben beziehen, gemischt- und getrenntgeschlechtliche Arten existieren, oder ob bei einem der beiden Beobachter ein Irrtum vorliegt.“

Verfasser hat nun in diesem Sommer einen Monat durch Beobachtungen über die Kopulation von *S. sticticum* angestellt. Seitenkopulation (von einer zur anderen Zelle desselben Fadens) und kreuzweise Kopulation (zwischen den Zellen zweier Fäden) konnten nicht beobachtet werden, wohl aber eine andere interessante Erscheinung, über die im folgenden berichtet werden soll.

Diese Beobachtungen scheinen *Sirogonium sticticum* als eindeutig gemischtgeschlechtlich erkennen zu lassen. Da die Beschaffung des Untersuchungsmaterials leider an eine kurze Zeitspanne während des Sommers gebunden ist, war es mir jedoch nicht möglich meine Untersuchungen im wünschenswerten Umfang durchzuführen. Die Fortführung der Arbeit und die Berichtigung etwaiger Irrtümer muß daher Untersuchungen des nächsten Sommers vorbehalten bleiben.

1) KNIEP, HANS: Die Sexualität der niederen Pflanzen, 1928.

### Material und Methode

Das Material, zahlreiche Sirogonien in Kopulation, wurde Ende Juni zufällig neben anderen Algen, die zu untersuchen ursprünglich beabsichtigt war, im Aquarium entdeckt, 10 Monate nachdem es mit diesen zusammen aus dem Fischteich Oike bei Himeji in Mitteljapan entnommen worden war. (Dem Wasser des Aquariums war KNOPSche Nährlösung nicht zugesetzt worden.) Schon Ende Juli waren keine Kopulationen mehr zu beobachten. Außer den Zygoten war der Inhalt der Zellen der Fäden bereits verkümmert und die Zellen zeigten sich von zahlreichen Oedogonien im Jugendstadium erfüllt. Jetzt, im Oktober, sind die Sirogonien völlig durch die ausgewachsenen Oedogonien verdrängt.

Um den Druck des Deckglases auszuschalten, wurden unter dessen Rand einige menschliche Kopfhare oder einige Deckglas-splitters geklemmt und um das Präparat vor dem Austrocknen zu schützen, wurde es in einem feuchten Behälter aufbewahrt.

### Beobachtung

Die Formen der steril bleibenden Zellen. Nach den Angaben der bisherigen Autoren enthält entweder der männliche Faden je eine lange und eine kurze steril bleibende Zelle, der weibliche jedoch nur eine einzige sterile Zelle (Textabb. 1, A, erste Form) oder (Textabb. 1, B, zweite Form); männlicher und weiblicher Faden enthalten also je nur eine steril bleibende Zelle. Das Auftreten dieser beiden Formen wird als regelmäßig bezeichnet. Wie jedoch Fig. 2 (Taf. 31) der vorliegenden Arbeit zeigt, kann auch im männlichen Faden nur eine einzige steril bleibende Zelle auftreten, während der weibliche Faden deren zwei aufweist, die in der Größe nicht verschieden sind (dritte Form).

Fig. 5 zeigt dann sowohl im männlichen, wie im weiblichen Faden je eine kurze und eine lange steril bleibende Zelle (vierte Form).

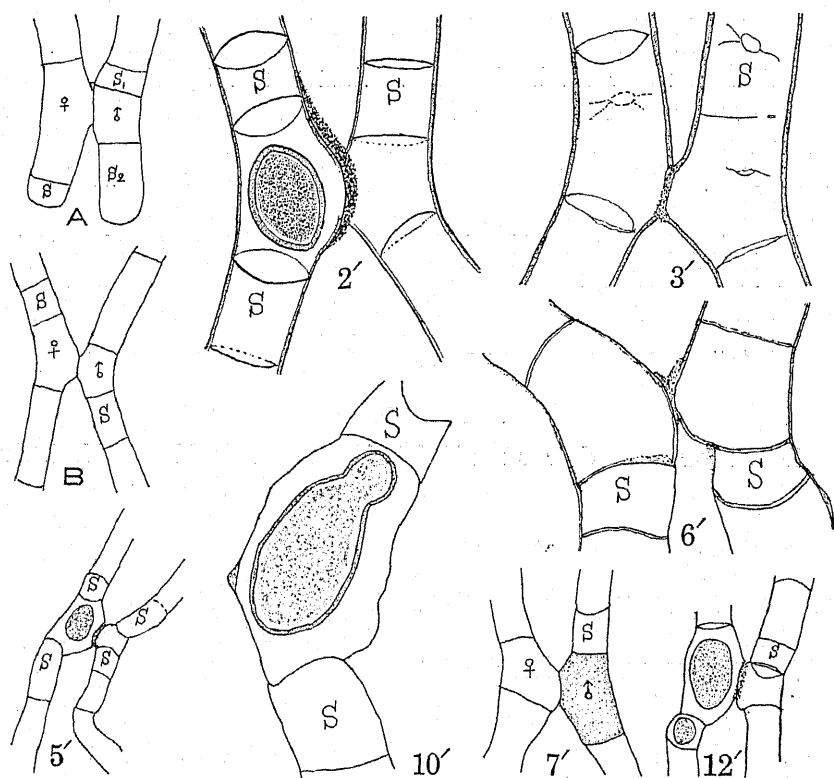
Fig. 6 schließlich läßt im männlichen, wie im weiblichen Faden je eine steril bleibende Zelle erkennen. Rein zahlenmäßig wäre damit der Fall 2 gegeben, jedoch besteht ein wesentlicher Unterschied in der Lage der Zellen (fünfte Form).

Textabb. 1, B stimmt mit Fig. 12 beinahe überein.

Außer diesen genannten Beispielen wurde noch eine große Anzahl anderer Formen und Gruppierungen der steril bleibenden Zellen beobachtet, z.B. eine größere Anzahl hintereinanderfolgender steriler Zellen.

Männliche und weibliche Gametangien. Nach den bisherigen Beobachtungen gehen aus dem männlichen Gametangium

nach zweimaliger Teilung zwei steril bleibende Zellen hervor, aus den weiblichen Gametangien wird nach einmaliger Teilung je eine steril bleibende Zelle abgetrennt. Deshalb ist das männliche Gametangium stets viel kürzer als das weibliche. (DE BARY, KNIEP, H., FRITSCH, F. E., OKAMURA, K., OLTMANN, F., WEST, G. S.) Nach



Textabb. 1. A. erste Form. B. zweite Form. Die Abbildungen 2', 3', 5', 6', 7', 10', 12' sind (die angegebenen Reihenfolge entsprechend) etwas schematisierte Darstellungen der auf die Tafel wiedergegebenen Photographien (Taf. 31, Fig. 2, 3, 5, 6, 7, 10, 12). S steril bleibende Zelle, ♂ männliche Gametangium, ♀ weibliche Gametangium.

G. M. SMITH entstehen sowohl männliches, wie weibliches Gametangium nach einmaliger Teilung. Auch in diesem Falle ist das männliche Gametangium kürzer als das weibliche. Auch nach den allgemein bekannten Abbildungen ist das männliche Gametangium kürzer als das weibliche. In meinem Falle jedoch ist im Gegenteil das männliche Gametangium sehr häufig viel länger, als das weibliche (Fig. 7 zeigt dies hinreichend deutlich, jedoch wurden sehr häufig noch ausgeprägtere Fälle beobachtet, von denen leider keine Photographie vorliegt). Die weiblichen Gameten haben nach den bisherigen Beobachtungen bauchig aufgetriebene, die männlichen schlanke Gestalt. Auch hier konnte ich gegenteilige Fälle beobachten.

So zeigt die Fig. 8 ein stark aufgetriebenes männliches Gametangium.

Gestalt der Zygoten. Die weiblichen Gametangien sind wie gesagt teils dick, teils schlank, teils lang, teils kurz. Die Zygoten zeigen dementsprechend teils rundliche teils elliptische Gestalt, manchmal treten auch abnormale Formen auf (Fig. 9 und 10). Fig. 10 zeigt eine abnorme Überwinterungsform, welche die Merkmale vorzeitiger, rasch wieder zum Stillstand gekommener Keimung zeigt. Man könnte zunächst glauben, daß man es mit einer Aplanospore zu tun habe, aber bei genauerer Betrachtung erkennt man aus der Nachbarschaft der steril gebliebenen Zellen und aus der Eintrittsstelle des männlichen Plasmas den Charakter der Zygote. Auch bei *Ulothrix* hat Verfasser früher schon eine ähnliche Form beobachtet und sie zunächst für eine Aplanospore gehalten. Die Beobachtung des vorliegenden Falles könnte auch Licht auf den bei *Ulothrix* beobachteten werfen. Doch das soll Gegenstand einer späteren Untersuchung sein.

Parthenogenese. Fig. 12 läßt eine deutliche Parthenogenese erkennen. Fig. 13 zeigt den häufig beobachteten Fall einer rundlich geformten, nicht näher bestimmbaren Substanz im Innern einer (steril bleibenden) Zelle. Vielleicht besteht eine Beziehung zwischen dieser letzteren Erscheinung und der deutlichen Parthenogenese einer steril bleibenden Zelle in Fig. 12. Wahrscheinlicher ist allerdings, daß es sich um ein Abbaustadium handelt.

Falls es sich bei der Fig. 11 auch um einen Fall von Parthenogenese handeln sollte, würde sie dartun, daß es außer der rundlichen Form der Fig. 12 auch bei den parthenogenetischen Zygoten eine zweite, längliche Form gibt.

Verbindungskanal. *Spirogyra punctata* CLEVE und ähnliche Arten unterscheiden sich von den gewöhnlichen *Spirogyra*-Arten, indem sie, wie *Sirogonium*, keine spiraligen Chloroplasten besitzen und bei der Kopulation wie *Sirogonium* steril bleibende Zellen bilden. Auch erfolgt bei der Kopulation wie bei *Sirogonium* die Annäherung beider Gameten durch knieförmige Biegung. Ein bemerkenswerter Unterschied besteht jedoch darin, daß ein deutlich ausgeprägter Verbindungskanal bei *Sirogonium* nicht gebildet wird. Danach besitzt der Verbindungskanal eine besondere Bedeutung als Unterscheidungsmerkmal.

Fig. 15 zeigt nun einen Fall von *Sirogonium*, bei dem lange vor der Kopulationszeit sich an einer Zelle eine Vorwölbung bemerkbar macht. Derartige Fälle wurden häufig beobachtet, teils derart, daß an mehreren aufeinanderfolgenden Zellen eines Fadens je eine solche Vorwölbung, alle nach der gleichen Seite gerichtet, auftritt, teils daß an den zwei gegenüberliegenden Langseiten einer und derselben Zelle je eine derartige Vorwölbung sich findet. Auf den

Figuren 3 und 4 zeigt das rechte Gametangium einen deutlich entwickelten Verbindungskanal. Das weibliche Gametangium (Fig. 8 links) zweigt eine verbindungskanalähnliche Vorwölbung, die jedoch nicht als echter Verbindungskanal anzusprechen ist. Möglicherweise besteht eine Beziehung zwischen solchen letzteren Bildungen und den Vorwölbungen in der Art der Fig. 15, denen demnach vielleicht ein geschlechtlicher Charakter zuzusprechen wäre.

Wenn solchen Vorwölbungen wirklich der Charakter von Verbindungskanal-Anlagen zukommt, wären vier Fälle zu unterscheiden:

1. (Fig. 2): Selbst die Andeutung eines Verbindungskanals ist nicht vorhanden.
2. (Fig. 8): Eine Vorwölbung, die als Andeutung eines Verbindungskanals aufgefaßt werden kann, ist bei der weiblichen Gamete entwickelt.
3. (Fig. 3 und 4): Ein Gamet zeigt einen deutlich ausgebildeten Verbindungskanal.
4. An Zellen, deren Geschlechtlichkeit noch nicht erkennbar ist, zeigen sich vorzeitig Ansätze zur Bildung von Verbindungskanälen.

Auf jeden Fall scheint sich hier ein stufenweiser phylogenetischer Übergang zu *Spirogyra* anzudeuten.

Die nähere Untersuchung der Umstände, unter denen die Bildung des Verbindungskanals vor sich geht, soll einer späteren Untersuchung des Verfassers vorbehalten bleiben.

Zeitpunkt der Bildung der steril bleibenden Zellen. Wie Fig. 14 zeigt, sind den steril bleibenden Zellen ähnliche Gebilde schon in einem sehr frühen Entwicklungsstadium zu beobachten. Wenn es sich hier um echte steril bleibende Zellen handelt, wären zwei Fälle der Bildung steril bleibender Zellen zu unterscheiden:

1. Vorzeitige Bildung, vor der Berührung der Gameten. (Fig. 14).
2. Bildung nach der Berührung der Gameten. (Fig. 3: die nach der Gametenberührung entstandene steril bleibende Zelle ist noch nicht völlig ausgebildet.)

Protoplasmakontraktion in den Gameten. Bei der Kopulation kontrahiert sich nach den Angaben vieler Autoren das Plasma des männlichen Gameten zuerst (Fig. 7 und 8). Nur diese Feststellung konnte durch meine Beobachtungen in vollem Umfang bestätigt werden.

#### Besprechung der Ergebnisse

Mit der Beobachtung, daß das männliche Gametangium immer kürzer als das weibliche und das weibliche immer dicker als das



männliche sei, stimmen meine Beobachtungen nur zum Teil überein. In einer Reihe von Fällen hat Verfasser geradezu das Umgekehrte beobachtet. Bei Kopulation in frühen Stadien kann daher mit absoluter Sicherheit über den männlichen oder weiblichen Charakter der Gameten nichts ausgesagt werden. Die genannten morphologischen Kriterien versagen hier. Aus diesem Grunde wurde über den Charakter der Gameten in den Fig. 3, 4 und 6 nichts ausgesagt. F. WEBER unterschied im Jahre 1929 männliche und weibliche Gameten von *Spirogyra* auf Grund der verschiedenartigen Plasmolyse, im Sinne seiner protoplasmatischen Pflanzenanatomie. Ob diese Methode auch im vorliegenden Falle anwendbar ist, konnte im vorliegenden Falle noch nicht geprüft werden. Wohl weil das morphologische Kriterium bisher als ausreichend betrachtet wurde, ist für *Sirogonium* eine derartige Untersuchung bis jetzt unterblieben.

In fortgeschrittenen Kopulationsstadien jedoch ist eine Unterscheidung der Gameten leicht möglich, da sich das Plasma des männlichen Gameten zuerst kontrahiert.

Trotzdem Seitenkopulation oder kreuzweise Kopulation nicht beobachtet wurden, möchte Verfasser doch auf Grund der stark fluktuierenden Geschlechtsmerkmale *Sirogonium* als gemischtgeschlechtlich bezeichnen.

### Zusammenfassung

1. Die morphologische Unterscheidung des Geschlechtscharakters der Gametangien von *Sirogonium* ist keine zuverlässige Methode.
2. Auch die zahlenmäßige und morphologische Berücksichtigung der steril bleibenden Zellen gestattet keine zuverlässige Bestimmung.
3. Bei jüngeren Kopulationsstadien kann daher mangels anderer Methoden bis jetzt keine Bestimmung des Gametencharakters vorgenommen werden.
4. Die Methode, den Gametencharakter bei fortgeschrittenem Kopulationsstadium durch Beobachtung der Plasmakontraktion zu bestimmen (das Plasma der männlichen Gameten kontrahiert sich zuerst), konnte durch die vorliegende Untersuchung bestätigt werden.
5. Auf Grund der bei dieser Untersuchung gewonnenen Gesichtspunkte ist *Sirogonium* als gemischtgeschlechtlich zu bezeichnen.
6. Die rundliche, längliche oder abnormale Form der Zygote ist durch die Gestalt des weiblichen Gametangiums bestimmt.
7. In steril bleibenden Zellen wurde Parthenogenese beobachtet.
8. Vorwölbungen der Zellen, welche vielleicht als Ansätze zu Verbindungskanälen zu deuten sind, wurden in frühen Stadien, richtige Verbindungskanäle in zahlreichen Fällen im Stadium der Kopulation beobachtet.

bachtet. Phylogenetisch sind vielleicht stufenweise die Übergänge zu *Spirogyra* gegeben.

9. Es kann nichts darüber ausgesagt werden, ob zwischen den kleinen Zellen früher Entwicklungsstadien und den späteren steril bleibenden Zellen im Stadium der Kopulation eine Beziehung besteht.

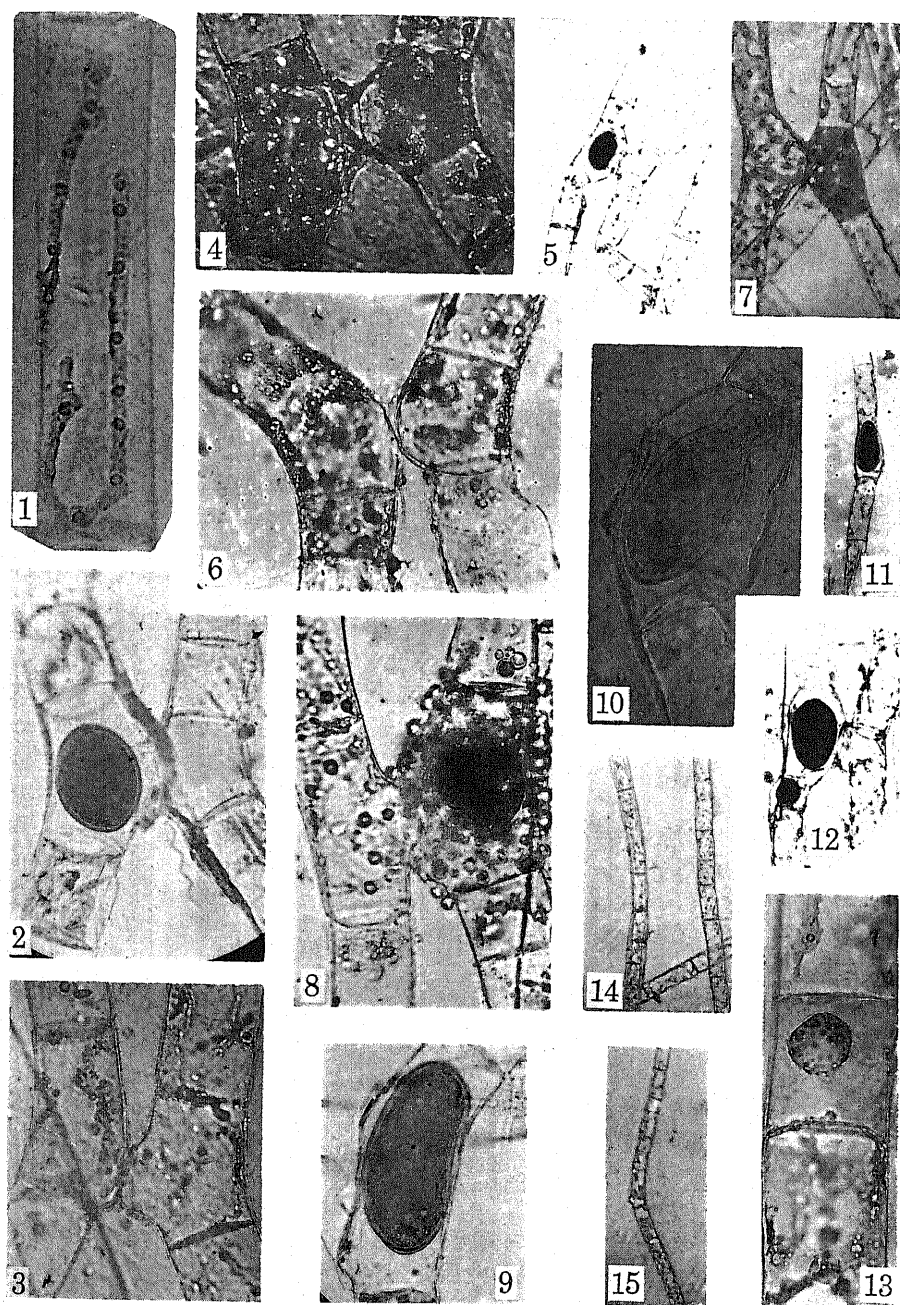
Diese kleine Arbeit möchte ich meinem hochverehrten Lehrer, Herrn Prof. honor. Dr. K. FUJII anlässlich der Feier seines 70. Geburtstags ergebenst widmen.

#### Literaturverzeichnis

- Correns, C. 1920. Die geschlechtliche Tendenz der Keimzellen gemischtgeschlechtlicher Pflanzen. Zeits. f. Bot. 12: 49-59 (2 Textabb.).
- Cunningham, B. 1917. Sexuality of filament of *Spirogyra*. Bot. Gaz. 63: 486-500 (Taf. 23-25).
- 1918. Cross conjugation in *Spirogyra Weberi*. Bot. Gaz. 66: 272-273.
- Czurda, V. 1925. Zur Kenntnis der Geschlechtsverhältnisse bei *Spirogyra*. Ber. d. Deutsch. Bot. Ges. 42: 441-444.
- De Bary, A. 1858. Untersuchungen über die Familie der Conjugaten. Leipzig.
- Edna, M. L. 1932. A Contribution to the life-history and cytology of two species of *Ulothrix*. Ann. Bot. 46: 711-724.
- Gay, F. 1884. II. Sur les Conjuguees du midi de la France. Bull. Soc. bot. de France 31: 331-342.
- Kniep, H. 1928. Die Sexualität der niederen Pflanzen.
- Okamura, K. 1930. Sôrui-Keitôgaku [Phylogenie der Algen] (Japanisch). Tokyo.
- Oltmanns, F. 1922. Morphologie und Biologie der Algen. 3 Bde. Jena.
- Smith, G. M. 1933. The fresh water algae of the U. S.
- Tröndle, A. 1907. Über die Kopulation und Keimung von *Spirogyra*. Bot. Zeitg. 65: 187-216 (13 Textabb.).
- Weber, Fr. 1924. Protoplasmaviskosität copulierender Spirogyren. Ber. d. D. Bot. Ges. 42: 279-284.
- 1925. Über die Beurteilung der Plasmaviskosität nach der Plasmolyseform (Untersuchungen an *Spirogyra*). Zeits. f. wiss. Mikrosk. 42: 146-156.
- 1929. Protoplasmatische Pflanzenanatomie. Protoplasma 8: 291-306.
- West, G. S. and Fritsch, F. E. 1927. A treatise on the British fresh-water Algae. Cambridge.
- Yamada, S. 1931. Sôrui [Algen] (Japanisch). Iwanami Kôza. Tokyo.

#### Erklärung der Tafel 31

- Fig. 1. Gewöhnliche vegetative Zelle. (377×).
- Fig. 2. Zygotenbildung. (298×).
- Fig. 3 und 4. Jüngerer Stadium der Kopulation. (beide 318×).
- Fig. 5. Zygotenbildung. (80×).
- Fig. 6 und 7. Jüngerer Stadium der Kopulation, in Abb. 7 rechts männlicher, links weiblicher Gamet. (318× bzw. 96×).
- Fig. 8. Jüngerer Stadium der Kopulation. Rechts männlicher, links weiblicher Gamet. (298×).
- Fig. 9 und 10. Zygoten. (beide 298×).
- Fig. 11. Parthenogenese? (54×).
- Fig. 12. Deutliche Parthenogenese und Zygotenbildung. (ca. 104×).
- Fig. 13. Unsicher, ob frühe Parthenogenese einer steril bleibenden Zelle (wie Abb. 12) oder Abbauerscheinung. (400×).
- Fig. 14. Lückenlose Reihe sterilbleibender Zellen in frühem Stadium? (48×).
- Fig. 15. Zelle mit Vorwölbung. (48×).



Kusunoki: Untersuchungen über die Geschlechtszellen von  
*Sirogonium sticticum* KUTZ.



## The Somatic Chromosomes of *Trillium*

By

Edward C. Jeffrey

The genus *Trillium* of Eastern Asia and North America has in recent years proved to be a cytological object extremely favorable for the investigation of general problems by reason of the extremely large size and relatively easy preparation of its chromosomes. Matsuura together with a number of other Japanese investigators and Nebel and Huskins in North America have occupied themselves with the study of the reproductive or meiotic chromosomes of the genus. These elements are favorable for study on account of their greater size but present the disadvantage of being involved in the nexus of conflicting and often highly controversial cytogenetical theories. This is the greater disadvantage as at the present time in cytogenetical investigation genetical theory seems to have far outstripped the firm basis of cytological facts. In the case of the somatic or more accurately the sporophytic chromosomes, we have to do with elements which have only half the volume of the reproductive ones and on account of their greater length are much less than half as thick as the corresponding reproductive chromosomes. In the case of structures approaching the verge of microscopical visibility this is certainly a handicap to be reckoned with. Further the greatest advances in the study of the extremely important minute organization of the chromosomes has been obtained by the agency of the smear method, only applicable to separate or loosely aggregated cells such as are frequently found in the case of the mother cells of the gonads or reproductive elements. The texturally closely united cells of the soma (sporophyte) can not be investigated by the method of smearing as under these circumstances they suffer serious injury. This signal disadvantage has made expedient the development of a method which would supply in the case of the cells of the body of the sporophyte in plants or the soma in animals a perfection of preservation comparable to the best results obtained in the case of the reproductive cells by the smear method. After many efforts this desirable end has been reached by a procedure described in subsequent paragraphs.

### Material

For the investigation here described four American species of *Trillium* have been employed, namely *T. grandiflorum*, *T. erectum*, *T.*

*undulatum* and *T. stylosum*. These four species are often grown for planting on estates in New England and it is thus easy to secure quantities of material with the minimum of effort. The species under consideration are also commonly kept dormant in cold storage so that there is considerable latitude in season. All the species named except *T. undulatum* have done well in our experience under greenhouse conditions. Over four hundred corms each of *T. grandiflorum* and of *T. erectum* have been studied in the present connection. In the case of the remaining two species one hundred specimens of each have been available. On receipt the corms are planted in shallow boxes about ten centimeters deep. The rapidity of development of the roots, which constitute the most favorable part of the plant for cytological investigations, depends on the temperature of the greenhouse and the season, since the new roots normally develop after the flowers have faded.

### Methods

As intimated above it has been found necessary to develop a special technique for the satisfactory preservation of the somatic cells. After much fruitless experiment the following method proved advantageous. It depends on two considerations. First of these is the rapid or rather instantaneous access of the best preservatives to the cells. This was attained by slicing the material into sections nearly thin enough for direct observation under the microscope. These thin slices are pushed off the blade of the knife rapidly into the preserving fluid. As soon as a moderate number of slices are in the fluid, they are exhausted under an air pump. An automobile tire pump in which the piston has been reversed and the check valve removed answers very well for this purpose and has the advantage of being readily transported either to the field or the greenhouse. After a sufficient amount of material has been accumulated the bottle is set aside in a cool place (a refrigerator serves very well) over night. It is then washed to remove the excess of preservative. Washing under running water is not necessary as a dozen or more decantations suffice, since it is not essential to remove the reagents entirely from the tissues. The material is then run up in grades of alcohol until 70 per cent is reached in which it may be stored indefinitely. Benda's fluid consisting of four volumes of chromic acid of one per cent-strength (to which is added one fifth of a per cent of glacial acetic acid) plus one volume of osmic acid of two per cent strength was used to advantage. This fluid in various individual modifications is currently used by cytologists at the present time where the best fixation is desired. In the case of the roots it is often more advantageous to



slice them longitudinally. This may be done in the manner of the older botanists by placing the root between thumb and index finger and slicing lengthwise one or more times.

Assuming that the material is in alcohol of seventy per cent, it is raised by degrees to 95 per cent. After remaining a few hours in strong alcohol it is transferred to a mixture of equal parts of alcohol and glycerine for half an hour or indefinitely. Beforehand a number of pieces of homogeneous cardboard 18 millimeters square are prepared. The cardboard should be free from paper pasted to either surface as is often found in ornamental board. The material is now transferred on a section lifter to the cardboard, care being taken not to spread it too thickly (not more than a millimeter or two in depth). The next step is to drop a six per cent solution of nitrocellulose in a mixture of alcohol (95 per cent) and ether over the cards. After this has dried slightly, pieces of paraffined paper cut the size of the cards are placed over the material and wrapped around in two dimensions by fine thread (50 to 60 gage) which is securely tied. The cards are now placed in 95 per cent alcohol to which about 10 per cent of chloroform has been added for the purpose of hardening the nitrocellulose. The cards are then placed in clean alcohol and thoroughly pricked with a fine needle. The holes should not be more than a millimeter apart. The cards are now freed of air by means of an air pump. The cards are now embedded in nitrocellulose after the manner described by Jeffrey (1) and Wetmore (2). Sections are cut on the sliding microtome, which may be stained in any desired manner as the matrix on nitrocellulose presents no hindrance to differential staining. The author has discarded the customary crystal violet in favor of double staining by iron alum hematoxylin and safranin. After subjection for a half hour or more to the action of iron alum, the sections after careful washing are transferred to a solution of one tenth of one per cent of crystallized hematoxyline, which may be kept indefinitely by the addition to saturation of phenol. After half an hour sojourn in hematoxylin they are bleached carefully under the microscope by a much diluted solution of iron alum (one half of one per cent answers very well). When the hematoxylin color is nearly gone, the sections are again very carefully washed to remove all trace of iron alum, which would cause early fading. They are left over night advantageously in extremely weak safranin, made by adding a single drop of an alcoholic solution of safranin to a watch-glass of distilled water. Next morning they are dehydrated by successive changes of absolute alcohol, to which a little chloroform has been added in order to prevent the softening of the nitrocellulose embedding mass. Three changes of absolute alcohol are sufficient



for dehydration and the amount of safranin retained depends on the rapidity of transfer through the several changes. Generally speaking it is advisable to decolor until the red tint has disappeared from the matrix and from the section. If this is carried too far the safranin is removed from the nucleus. The combination of safranin and hematoxylin reveals an unusually rich detail of nuclear structure. Naturally the nucleus is best preserved in those cells nearest to the fixing reagent, but if the slices are made thin enough the cells are in general well preserved, particularly the nuclear structures. The sections are now cleared in xylol or benzol and mounted in Canada balsam or Dammar resin dissolved in xylol or benzol as the case may be. Benzol offers the not inconsiderable advantage of much more rapid hardening of the mounted preparations. The mounts are flattened and cleaned in the usual manner in preparation for study under the oil immersion. In the present investigation Zeiss 3 mm. aperture 1.4, 1.5 mm. aperture 1.30 and Leitz 3 mm. aperture 1.4 and Leitz one sixteenth have been employed. These were controlled by Zeiss and Leitz 2 mm. aperture 1.4 lenses.

The large size of the chromosomes makes observations in the case of *Trillium* particularly favorable. *T. grandiflorum* has the

largest chromosomes of the four species studied but all were suitable for study. Figure 1 shows a metaphase from a longitudinal section of the root of *T. grandiflorum*. The chromosomal elements are not only divided into two daughter chromosomes which are quite distinctly separated from one another, but these in turn are subdivided into two daughter chromatids (chromonemata) which are still retained within the body of the daughter chromosome. These two secondary chromonemata or chromatids do not acquire complete individuality until the next following metaphase. It will be seen that the primary daughter chromosomes are in some cases coiled around one another

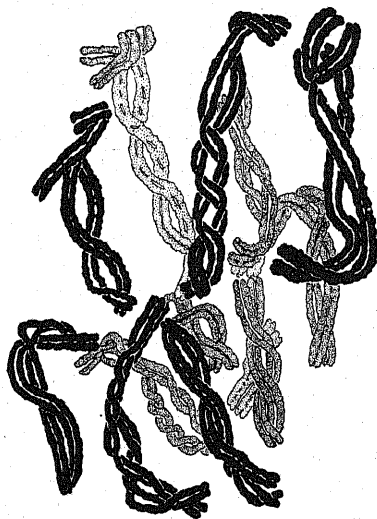


Fig. 1. Metaphase of *Trillium grandiflorum*.  $\times 3000$ .

although this feature, which is marked in the prophases preceding the metaphase has to a large extent disappeared. In other words the primary daughter chromosomes have become largely straightened out

and with the onset of the anaphase this condition becomes the rule. An interesting condition is presented in the case of the subdivision of the primary daughter chromosomes. The line of separation tends towards the curved or rectilinear. The marked spiral condition, which as will be seen immediately, marks the anaphase, is absent. It is at this stage that the subdivision of the daughter chromosomes is effected. Consequently the problem presented by the appearance of two contrarywise enwrapped spirals at a later stage is resolved by a study of the origin of the spirals, since the chromatids are originally not characterized by the presence of the so-called gyres, which as we shall see are of later origin.

Figure 2 A shows the definitely developed anaphase in the case of the root of *T. grandiflorum*. Gyres are now strikingly present

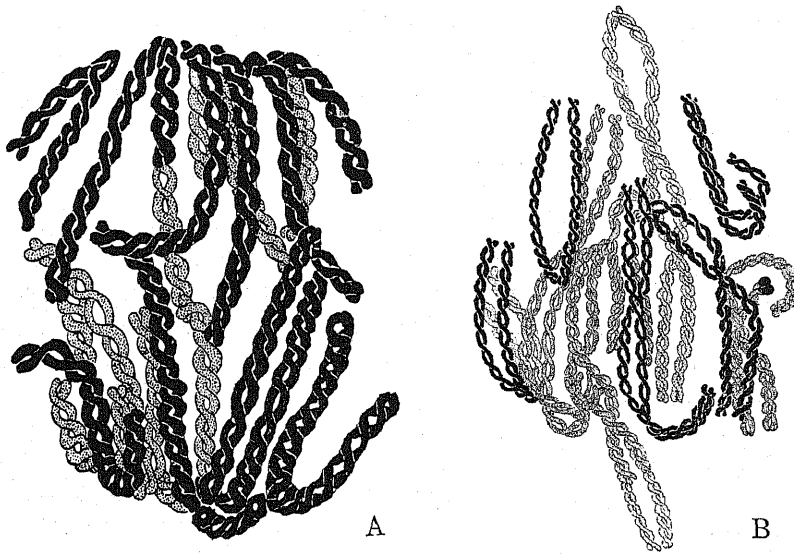


Fig. 2. Anaphases, A. *T. grandiflorum*,  $\times 3000$ ; B. *T. stylosum*,  $\times 3000$ .

and are the result of the spiralization in opposite directions of the two chromatids or chromonemata of the daughter chromosomes following the metaphase. The spiralization of the chromonemata in the anaphase is beyond reasonable doubt a contraction phenomenon. It is just as easily explainable or unexplainable as the spiralization of the flowering axis of *Nymphaea*, *Vallisneria* and many other water plants for the purpose of drawing the fruit down below the surface of the water. As the anaphase verges into the telophase the two involved spirals of each anaphase chromosome become violently contracted so that details of structure are for a time more or less completely obliterated. Gradually the contraction is relaxed and the

reversely spiraled chromatids are again visible. The spirals are now broader and the so-called gyres are closer together. This stage is followed by one in which the original pair of gyrating chromatids, while for the moment retaining their joint individuality, gradually undergo a process of loosening up in which the spiral structure finally disappears. Next the individuality of the chromosomal elements is lost in the apparently granular or reticular resting stage. Nucleoli now begin to appear in the substance of the resting nucleus. As a prelude to renewed activity, the nucleoli again disappear and chromosomal organization is once more established. The spireme is at first apparently continuous and shortly is obviously double once more, with its two chromatids or chromonemata reversely wound around one another, thus resuming the double organization and strong spiralization, characteristic of the anaphase. Gradually the prometaphase appears and with it the individuality of the ten chromosomes is once more established. Gradually the spiralization of the chromosomes becomes obliterated as the metaphase is fully established. The metaphase chromosomes having now become considerably straightened out divide longitudinally into two relatively rectilinear or curvilinear halves, which later as the metaphase passes into the anaphase become strongly contracted in the form of reversely orientated spirals.

It is thus obvious that the somatic chromosomes present a strong contrast to the reproductive chromosomes and those of the gametophytes as described by Huskins (3), Matsuura (4) and Jeffrey (5) as well as by many others. The diversity of the mode of mutual relation of the chromatids in the two kinds of cells is the striking feature, for in the reproductive cells and the gametophytes of *Trillium* the spirals in general run in the same direction, whereas in the sporophytic (somatic) cells they run in reverse. The present writer has observed the same contrast in the case of *Tradescantia*, where there is likewise parallelism in the chromatids of the reproductive elements and reverse order in the case of the somatic cells. Apparently the mutual orientation of the twin chromatids is not a matter of great importance as in the majority of known cases they are reversely orientated even in the reproductive cells. This is true for example of *Lilium*, *Allium* and *Vicia faba*.

Figure 2, B shows the smaller chromosomes of the late metaphase of *T. stylosum*. Although the chromosomes still show to some extent the paired condition characteristic of the metaphase, the contained pairs of daughter chromatids present the strongly spiralized condition which is so marked a feature of the anaphase. The figure in fact might well be characterized by the appellation metanaphase. Although the daughter chromosomes of the anaphase are clearly

separate and distinct the anaphase chromatids are still lodged in a common chromosomal body.

At this stage it will be convenient to refer to the appearance presented by the chromosomes in transverse section. In the metaphase we see two daughter chromosomes; distinct from one another and lying side by side. If each of the daughter chromosomes is likewise examined in transverse section it appears to have a light center of lumen surrounded by chromosomal material. It is possible to note in favorable cases that the surrounding cordon of chromatin is divided into two. This represents the cross section of the two chromatids which have originated already in the daughter chromosome following the metaphase. Later in the anaphase the individuality of the daughter chromatids (not to be confused with the daughter chromosomes) becomes still more clearly developed.

In this connection it becomes possible to make an apparently plausible suggestion in regard to the nature of the so-called chromomeres, which have been identified by the cytogeneticists as the morphological representatives of the highly theoretical concepts, known as genes. The present writer has not been able to identify any structure in the chromosomes of active nuclei corresponding to chromomeres. The structures so designated appear in the light of the study of apparently more perfectly preserved material, to be optical illusions corresponding to the gyres of chromatids or to their points of crossing where reversely oriented. If such turns out to be a true description of their nature, they can no longer be used as a basis of cytogenetical speculations in regard to genes, chiasmotypy, etc., etc.

It will be advantageous at this stage to present the evidence of photomicrographs. Figure 3, A shows a moderately magnified photomicrograph of the metaphase in the somatic cells

of *T. grandiflorum*. The presence of the daughter chromosomes resulting from division can be distinguished and it is obvious that to some extent the daughter chromosomes are still spirally involved with one another in opposite directions.

Item B of the same figure

represents a more highly magnified view of the same metaphase, in

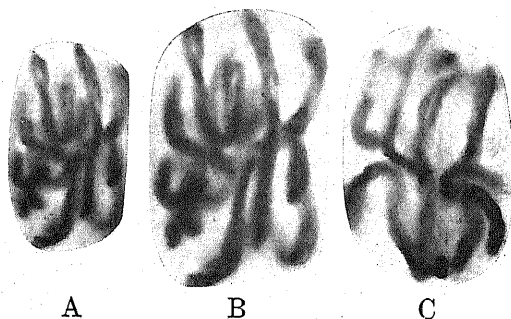


Fig. 3. Metaphases of *T. grandiflorum*, A.  $\times 1000$ ; B.  $\times 1500$ ; C.  $\times 1500$ .

which the features are correspondingly clearer. In C is shown another metaphase. In the second upper pair of chromosomes from the right of the illustration, it is possible to make out that the daughter chromosomes are already subdivided into chromatids or chromonemata, which instead of showing strong spiralization as they do at a later stage represent a condition of parallelism, following their origin from the longitudinal division of each daughter chromosome.

In Figure 4, A is shown under a considerable degree of magnification the anaphase. Even with this magnification it is obvious that the

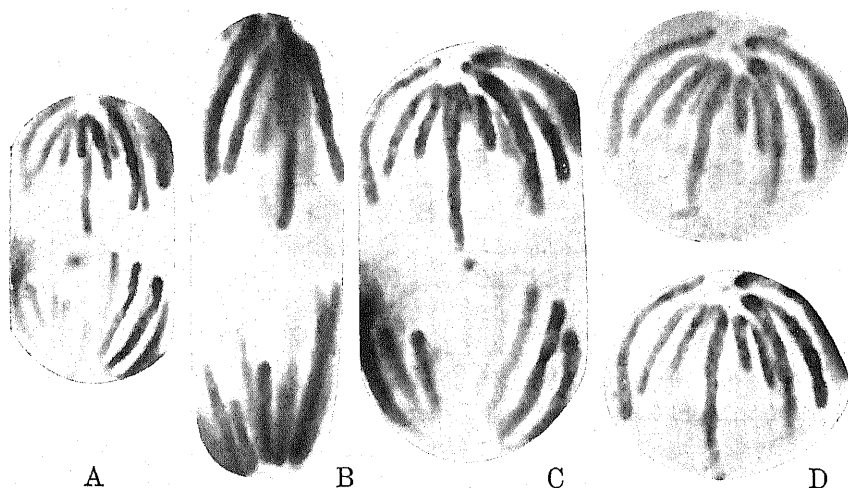


Fig. 4. Anaphases, *T. grandiflorum*, A.  $\times 1500$ ; B.  $\times 3300$ ; C.  $\times 3000$ ; D.  $\times 3300$ .

chromatids are spiral in their organization both in the upper and lower groups of chromosomes. In B is shown a very highly magnified picture of the late anaphase. The spiral nature of the chromatids can be distinguished in the second chromosome from the left of the upper group appearing in the illustration. In C appears another highly magnified view of an anaphase in its broad aspect. The chromosomes here again show spiral structure both in the lower and upper groups. In D are shown two views at different foci of the upper group of chromosomes. The structures in question might by some be interpreted in terms of the alveolar in contrast to the chromonemal organization of chromosomes. The extreme magnification (over 3000) makes sharp focusing of stained structures difficult. It should be emphasized in this connection that the diameter of the sporophytic chromosomes in *Trillium* is only about one fourth that of the reproductive chromosomes, due to their halved volume and much greater length.

### Conclusions

It should be emphasized in this connection that the best possible technical manipulation is necessary in dealing with the extremely minute structures involved in the organization of the nucleus, particularly the somatic nucleus and its activities in division. It is considered that the improvements in methods described at the outset of the present communication throw a considerably greater light on the minute organization of the nucleus, a subject of extremely great biological importance. They further give promise of bringing about a much to be desired clarification of the mutual boundaries between cytology and genetics. There apparently exists at the present time a deplorable tendency to base too great genetical conclusions on too imperfectly known cytological structures.

It further seems obvious that the continued study of the somatic chromosomes with improved methods, in spite of their relatively small size, will be of the greatest advantage to cytology. This is true because the course of events in the case of the reproductive chromosomes has become extremely involved with cytogenetical hypotheses. Since the course of kariokinesis in the soma is essentially the same as in the reproductive mother cells there is good reason to hope that we shall before long have a clear and more satisfactory conception of the nature of the reduction division, sex chromosomes etc.

It is now obvious that the chromatidal or chromonemal organization of the chromosomes is in the way of being more firmly established and that the alveolar hypothesis must be relegated to the limbo of outworn hypotheses. The chromomere as a constituent of active chromosomes appears to lack firm foundation and can scarcely be considered in the light of the morphological basis of the gene.

### Summary

1. In spite of their smaller size the somatic chromosomes are well worthy of investigation.
2. Improvements in cytological technique are here described which make possible as accurate fixation of the somatic cells as that afforded by the best procedure in the case of the smear method.
3. The somatic cells on account of the difficulties of fixation have not been made to bear the extreme load of theory which has been imposed on the reproductive elements.
4. The essential organization of the dividing nucleus is chromatidal or chromonemal.
5. In the metaphase the chromatids, originating in the anaphase or late metaphase of the preceding divisions, become separated from one another and divest themselves of their spiral involvements.

6. At this stage they subdivide longitudinally.
7. Later as the metaphase passes into the anaphase the two chromatids of each daughter chromosomes become spirally involved in opposite directions.
8. This involvement reaches its extreme condition in the fully developed anaphase.
9. The chromatidal pairs undergo strong contraction in the telophase.
10. This condition is followed later by a loosening in which the pairs of chromatids again reveal their identity. At this stage their spiral structure is no longer obvious.
11. Their identity is apparently lost in the resting stage.
12. Nucleoli make their appearance in the resting nuclei.
13. The revival of nuclear activity brings with it the disappearance of the nucleoli and the reestablishment of the filamentous organization.
14. The nuclear filament is clearly double and consists of two chromatids reversely coiled.
15. The spiralization gradually disappears with the development of the metaphase.
16. The so-called chromomeres are apparently optical illusions based on the misinterpretation of gyres or contragyres resulting from imperfect preservation.
17. The study of somatic divisions with improved methods is apparently destined to throw a new fundamental light on the nature of the meiotic or reduction division.

#### Literature

- (1) Jeffrey, E. C. Technical contributions, Bot. Gaz. 86, no. 4, Dec., 1928.
- (2) Wetmore, R. H. The use of celloidin in botanical technic, Stain Technology, 7, 37, 1932.
- (3) Huskins, C., Leonard and Smith, Stanley G. Meiotic Chromosome structure in *Trillium erectum*, Ann. Bot. 49, No. 193, Jan., 1935.
- (4) Matsuura, H. Chromosome studies on *Trillium kamschaticum*, Cytologia 6, pps. 270-280, 1935.
- (5) Jeffrey, E. C. The structure of chromosomes, Science, 1936, *Ined.*



## The Chromosomes of Two Elasmobranch Fishes <sup>1)</sup>

By

**Sajiro Makino**

Zool. Inst., Hokkaido Imp. Univ., Sapporo

(With 21 Text figures)

The Elasmobranchs, or sharks and skates, were the classical objects of investigations in spermatogenesis and oogenesis, which were carried out at the end of the last and in the beginning of the present century, a number of papers having been published in this field by pioneer investigators (cf. Oguma and Makino '32). The majority of the investigations were directed towards elucidation of the maturing phenomena in the sperm and egg cells, and, consequently, the chromosome studies were, at that time, of secondary importance to the earlier investigators, who, at best, made only an approximate calculation of the chromosome number in these animals. Probably due to the fact that the Elasmobranchs are comparatively difficult material for cytological study, not much progress was made in this direction. Whilst circumstantial works on Teleosts, another group of Pisces, analyzing the number, shape and behaviour of their chromosomes carried out with advanced karyological technique continued to appear from time to time (Winge '22, Geiser '24, Foley '26, Vaupel '29, Iriki '32, a, b, Friedman and Gordon '34, Ralston '34, Makino '34 a, b, '37, Prokofieva '34, Bennington '36). Under these circumstances, an accurate survey of the chromosomes of Elasmobranchs becomes increasingly necessary in order to establish chromosomal relation with Teleosts on the one hand, and, on the other hand, to enable us to have a glimpse of the course of karyological evolution in vertebrate animals. The present paper dealing with the chromosomes of two species of the Elasmobranchs, the shark and the skate, has been prepared with this point in view.

The work has been carried out under the guidance of Prof. Kan Oguma to whom the author wishes to express his best thanks.

### 1. The Skate, *Raja meerdervoortii* Bleeker

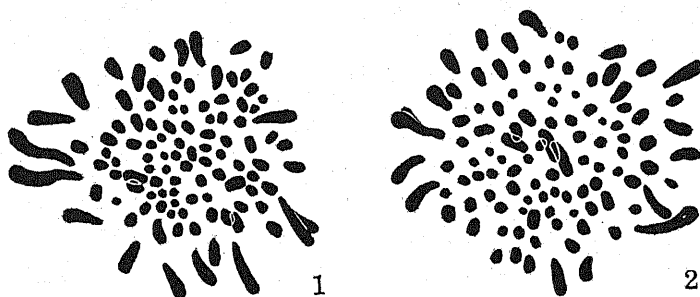
The skate herein dealt with is a common representative of the Rajidae belonging to the suborder Batoidei, which is abundant on the coasts of Japan, especially northward. The material on which the

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1) Contribution No. 110 from the Zoological Institute, Faculty of Science, Hokkaido Imperial University.

following observations are based, consisted of the testes of two specimens obtained at the Asamushi Marine Biological Station, Aomori-Ken, by the author in May 1934, through the courtesy of Prof. S. Kokubo, and the author tenders his thanks to him for the kindly help. The testes were dissected out in the living state, cut into small fragments, and dropped into Champy's mixture. Sections were stained with Heidenhain's iron haematoxylin. In the preparations thus made the chromosomes were preserved with sufficient distinctness to admit of an accurate survey.

*The Spermatogonial Chromosome:* Due to the considerably large size of the cell in this species, as contrasted with the Teleosts, the study of chromosomes could be carried out with facility in spite of the large number of chromosomes. The



Text figs. 1-2. *Raja meerdervoortii* Bleeker. Metaphase groups of the spermatogonia, 104 chromosomes in each.  $\times 3400$ .

spermatogonial chromosomes were examined in several clear metaphase plates, in order to confirm the number and form of chromosomes. Text figs. 1 and 2 are polar views of the spermatogonial metaphase which were drawn from well preserved, large equatorial plates. By carefully counting such clear figures it was decided without any difficulty that the number of chromosomes in a spermatogonium is 104. The chromosomes are seen at the metaphase in the usual radial arrangement forming a typical rosette; the larger chromosomes, which assume the rod shape in telomitic kind, tapering at their inner ends and numbering about 20, occupy the outer circle of the spindle, with the smaller ones of granular or spherical form in the central area.

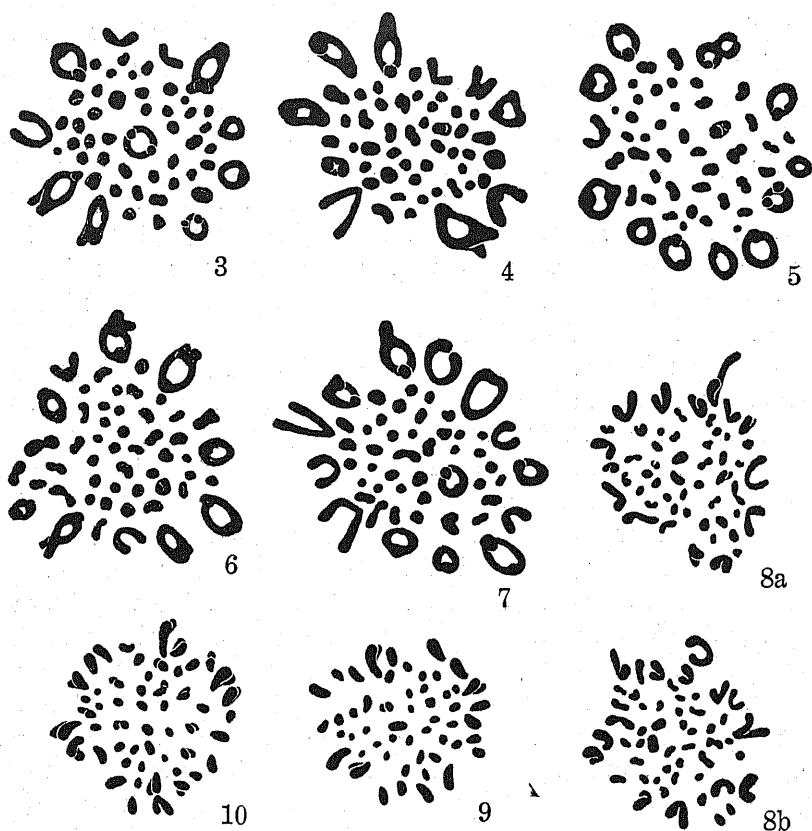
According to the apparent featural resemblance in size and form, the homologous chromosomes could be easily distinguished among the larger ones which vary in length in graded order, while the smaller ones could not be paired equally easily inasmuch as all such chromosomes assume uniform shape and size without showing any deviation which could be utilised as a character for identifica-

tion. After examination in this manner it can only be said that no unpaired elements could be identified among the larger chromosomes so far as their morphological characters admitted of a comparison. As to whether any kind of chromosomes peculiar in respect of shape and behaviour is contained in this complex or not, will be established only when they are seen in the anaphase stage in the first division.

*The Spermatocyte Chromosome:* The number of tetrads which arrange in the metaphase equatorial plate of the primary spermatocyte is without exception 52 in the haploid one; they usually spread themselves out radially, every one of them being well apart at nearly equal intervals, so as to form a rosette (Text figs. 3-7). The larger tetrads which lie at the marginal zone of the equatorial plate appear ring-like lying horizontally or thick V-shaped when the distal ends of chromatids remain unfolded, whilst those of smaller size lying scattered in the central area of the equatorial plate are like thick dots or bipartite in shape. The ring tetrads generally assume the form of simple rings and their distal ends are entwined into an elongated, heavy knot, sometimes split horizontally, while at the proximal ends where the spindle fibres are attached a globule is, in most cases, formed as a distinct structure. Sometimes the tetrad is composed of two connected rings when the distal limbs of the ring-tetrad is sufficiently elongated to make one more ring possible. One such example is found in one of the peripheral tetrads shown in Text fig. 5. At any rate, judging from the structural condition of the univalent chromosomes, it is reasonable to think that these horizontal ring tetrads with terminal fibres may belong to the same category as those commonly occurring in the Orthoptera and those recently reported in certain species of Mammalia and Reptilia.

Generally speaking, it is considered that one of the critical stages to examine to find out whether the tetrad is composed of equal or unequal component halves, is the anaphase of the first division when they segregate into constituent elements. On this account, the behaviour of chromosomes during the anaphasic migration in the first division was accurately studied. In this division the tetrads become separated into two composing halves along the plane parallel to the equatorial plate. The larger horizontal ring tetrads are split into two identical, single V-shaped dyads by the plane of conjugation as in the Orthoptera. The smaller ones are also divided into equal components, each halves appearing in the form of dumb-bells, dots or single small V's. Text fig. 8 a and b represent the polar views of the anaphasic sister complexes of chromosomes in the first division as observed in a single section, in each of which 52 chromosomes correspond with each other pairwise admirably. Thus, it is concluded

that all of 52 chromosomes of the primary spermatocyte are to be regarded as ordinary tetrads and no particular tetrads showing size difference between the constituent halves exist in so far as shown by the first division.



Text figs. 3-10. *Raja meerdervoortii* Bleeker. 3-7, metaphase groups of the primary spermatocytes, 52 tetrads in each. 8a-8b, sister chromosome groups in anaphase of the first division. 9-10, metaphase groups of the secondary spermatocytes, 52 dyads in each.  $\times 3400$ .

As naturally expected from the mode of separation of tetrads in the first division, every metaphase plate of the secondary spermatocytes invariably shows 52 dyads (Text figs. 9-10). They correspond in form, size and arrangement to those observed in the spermatogonium, though the garniture shows only the complete half set of what is found in the latter. The larger dyads which arrange themselves radially on the peripheral circle of the spindle, with their tapering ends directed towards the centre enclosing the smaller ones, assume the shape of vertical Vs, the two constituent rod-shaped

monads opening at the distal end along the radii of the equatorial plate, while their proximal ends are still in contact. No chromosomes unusual in shape or behaviour were found in the second division.

*Remarks:* On looking up the literature, references to two classical papers by Moore ('95) and Farmer and Moore ('04) on the chromosomes of *Raja* were found. According to these papers, the diploid number of chromosomes in the male is 24 and the haploid number is 12 in both *Raja macrorhynchus* and *Raja maculata*. These results however, cannot be accepted un-questioned. The chromosome number in the species above mentioned is astonishingly low as compared with the results obtained by me in *Raja meerdervoortii*. It is doubtful whether these differences in the chromosome numbers can be said to be due to specific differences. The present author's experience does not warrant such a conclusion. Moreover, the figures given in these papers by Moore and Farmer are not absolutely convincing. For these reasons it seems better to leave out of consideration these results in our present discussion. Kastschenko ('90) reported about 50 chromosomes in the first maturation division of the egg of *Torpedo ocellata*, a species of electric ray belonging to a different family—Torpedidae—but to the same suborder Batoidei.

It is interesting to note that the chromosome complex in the present material is so complicated as to constitute a singular example in piscine chromosomes. Generally, the chromosomes of Teleosts are isomorphic in the complex, i.e., the chromosomes uniformly assume the shape of dots or short rods, though a few exceptional conditions are recorded in *Umbra* (Foley '26), *Oncorhynchus* (Makino, '37), and *Salmo* (Prokofieva '34). Among the vertebrates whose chromosomes have been studied, one finds a parallel case in some species of lizards and geckos in so far as the complex of chromosomes is concerned (cf. Matthey '31, '33; Nakamura '32, '35).

The chromosome number of 104 in *Raja meerdervoortii* is the highest one so far recorded for any vertebrate. This number is represented in the carp, *Cyprinus carpio* (Makino '34, b) in which, however, the complex is isomorphic, all the chromosomes being uniformly of granular shape.

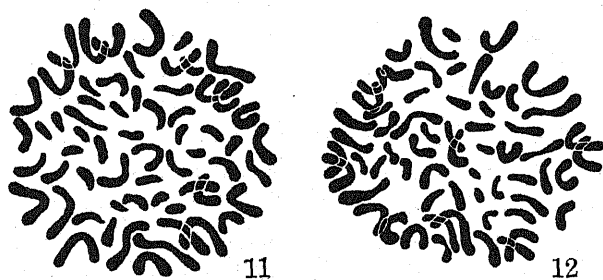
## 2. The Shark, *Squalus suckleyi* (Girard)

The shark, *Squalus suckleyi*, which is generally known as the Pacific dog fish, is distributed over the arctic and subarctic regions of the Pacific coast and abounds on the northern coasts of Japan. According to Sato ('35), the Pacific dog-fish breeds at every season

throughout the year in the coastal waters of Hokkaido. It belongs to the family Squalidae of the suborder Tectospondyli, a suborder different from the Batoidei to which *Raja*, the former material, belonged.

The testes from two individuals comprise the material for the present study, which were netted from the waters adjoining the Akkeshi Marine Biological Station, Hokkaido, in June 1934. They were operated upon and fixed immediately after capture on board the fishing boat. For fixation, Champy's mixtures, in its original strength, and also diluted with an equal part of distilled water, were employed, the former having proved to be the better of the two. Sections were cut 12 micra thick and stained with Heidenhain's iron haematoxylin. Here the author wishes to express his cordial thanks to Dr. Shinichi Sato with whose kind cooperation the present material was obtained.

*The Spermatogonial Chromosome:* On account of the germ cells being considerably advanced in the process of maturation



Text figs. 11-12. *Squalus suckleyi* (Girard). Metaphase groups of the spermatogonia, 62 chromosomes in each.  $\times 4200$ .

in the testes, early, large spermatogonia of the primary order could not be observed, but the material was good enough for the morphological study of chromosomes in the late spermatogonia of

small size. The diploid number observed in the spermatogonial metaphase was invariably determined to be 62, as represented in Text figs. 11-12. Though the shape of individual chromosomes was not conclusively determined in this study, it is clear from the accompanying figures that the diploid garniture is made up of various kinds of atelomitic chromosomes with median or submedian fibre attachments, which lie on the marginal circle of the equatorial plate with their apices towards the axis of the spindle and number nearly 20, or more, in an rough calculation, while the remaining chromosomes of probably telomitic kind in respect of fibre attachments lie scattered in the central area enclosed by the former. The chromosomes of the latter group vary in size and shape, some being slightly curved rods with taper ends and some being bent to a considerable degree resulting the apparent V's. That these chromosomes

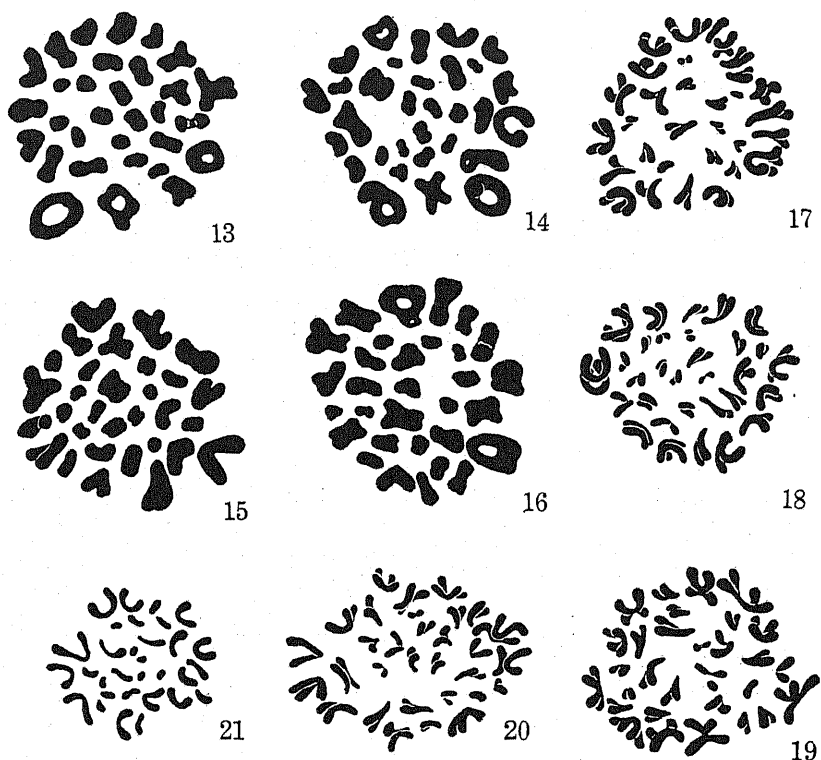
are in reality of the telomitic kinds is proved by an examination of well preserved metaphase chromosomes of the secondary spermatocyte in which the individual chromosomes show their definite shape to a great extent even in the reduced state.

*The Spermatocyte Chromosome:* In the metaphase equatorial plate of primary spermatocyte 31 tetrads of various size and shape are observable, as the result of the coupling of 62 univalents into pairs (Text figs. 13-16). They are well defined from one another in absolute clearness, but owing to unsatisfactory preservation the shape of the individual chromosomes is too poorly defined to make it possible to verify the relationship to the univalent chromosomes in the spermatogonium. Still it is possible to recognize in these figures that most of the larger tetrads lying at the periphery structurally suggest themselves to be provided with atelomitic fibre attachments, thereby showing that they must be the descendants of the pairs of V-shaped chromosomes in the spermatogonium. Among them one tetrad, which is possibly derived from the longest pair of rod-shaped chromosomes found in the diploid group, invariably appears in the distinctive form of a horizontal ring in every equatorial plate. In order to solve the question whether any tetrad composed of unequal parts occurred or not in the garniture, which could not be established from observations of the metaphase polar view, the spindle in process of the separation of chromosomes was studied in profile. The separation of chromosomes is nearly simultaneous and there is no element showing asymmetrical separation or extraordinary behaviour. Hence, it is clear that every tetrad consists of two dyads with equal valency.

A number of dividing figures of the secondary spermatocytes, which are sufficiently helpful in enabling one to determine the number of chromosomes, were fortunately obtained in the sections. Out of them, four figures of the metaphase in the polar view are given in Text figs. 17-20, every one of which contains 31 dyads constantly in the usual arrangement. At the periphery of the spindle are situated larger dyads with atelomitic and telomitic fibre attachments in radial direction, while in the central region smaller ones with telomitic attachments predominate. Although it is difficult to compare morphologically the individual chromosomes of the secondary spermatocyte with those of the spermatogonium, the garniture apparently corresponds to one half of set found in the spermatogonium; that is, nearly 10 atelomitic V-shaped ones and the rest with apparently telomitic attachments of various lengths constitute the garniture. All the dyads distinctly show a dual nature at the metaphase, being made up of two monads appearing at different levels in



the polar view. In the ensuing division they all split along the equatorial plane into two identical monads. Text fig. 21 shows one of the sister complexes when observed in the anaphase of the second division en face, in which the individual monads correspond in their shape to the dyads found in the secondary spermatocyte metaphase with absolute clearness.



Text figs. 13-21. *Squalus suckleyi* (Girard). 13-16, metaphase groups of the primary spermatocytes, 31 tetrads in each. 17-20, metaphase groups of the secondary spermatocytes, 31 dyads in each. 21, one of sister complexes in anaphase of the second division, 31 monads.  $\times 3400$ .

*Remarks:* Referring to the literature there are seven classical publications in which the chromosomes of the sharks have been described to a greater or less extent (cf. Oguma and Makino '32). Most of these papers are devoted to comprehensive descriptions of spermatogenetic and oogenetic processes. No special interest was taken in the number and form of chromosomes by these pioneer workers, probably due to the fact that the chromosomes had not been preserved adequately by means of the classical cytological technique, then in vogue, to make their precise study possible. On the

whole, therefore, their results embody no accurate accounts of the chromosomes and, it is quite impossible to discuss their data in the light of modern karyology. Still, there is, among them, a noteworthy paper by Schreiner ('07) which dealt with the chromosomes of *Spinax* (*Etmopterus*) *niger* belonging to the family Squalidae. Though the number of chromosomes had not been exactly settled, as he does the approximate number to be 60-70 in the spermatogonium, it is noteworthy that the chromosome complex of *Spinax niger* shows a close resemblance to the present species, *Squalus suckleyi*, suggesting specific relationship from the point of view of the form and number of chromosomes. And, judging from the evidences obtained from the present study of *Raja* and *Squalus* and the earlier work of Kastschenko ('90) on *Torpedo* and of Schreiner ('07) on *Spinax*, it may be concluded that the chromosome constitution of Elasmobranch fishes is generally not simple but a complicated one, with a rather high number of chromosomes.

### Summary

1. The number of chromosomes in *Raja meerdervoortii* Bleeker (Rajidae) is 104 in diploid and 52 in haploid. The diploid complex is composed of telomitic rod-shaped chromosomes, numbering about 20 disposed at the periphery of the spindle, and the remaining granular ones which always lie in the central space enclosed by the former.

2. The chromosome complement of *Squalus suckleyi* (Girard) (Squalidae) is 62 in diploid and 31 in haploid. The diploid garniture consists of several types of about 20 atelomitic chromosomes lying at the outer zone of the equator and the remaining ones, which are rod shaped with probably telomitic fibre attachment, lie in the central area.

3. In both of these cases, the author could not detect any chromosomes throughout the course of the meiotic divisions which from its shape or behaviour could be characterised as the heterochromosome.

### Literature

- Bennington, N. L. 1936. Germ cell origin and spermatogenesis in the Siamese fighting fish, *Betta splendens*. Journ. Morph., 60: 103.
- Farmer, J. B. and J. E. S. Moore 1904. On the meiotic phase in animals and plants. Quart. Journ. Micr. Sci., 48: 489.
- Foley, J. O. 1926. The spermatogenesis of *Umbra limi* with special reference to the behavior of the spermatogonial chromosomes and the first maturation division. Biol. Bull., 50: 117.
- Friedman, B. and M. Gordon 1934. Chromosome numbers in Xiphophorin fishes. Amer. Nat., 68: 446.

- Geiser, S. W. 1924. Sex ratio and spermatogenesis in the topminnow, *Gambusia holbrooki* GRD. Biol. Bull., 47: 175.
- Iriki, Sh. 1932a. Preliminary notes on the chromosomes of Pisces. 1. Proc. Imp. Acad. (Tokyo), 8: 262.
- 1932b. On the chromosomes of *Aplocheilichthys latipes*. Sci. Rep. Tokyo Bunrika Daigaku, Sec. B, 1: 127.
- Kastschenko, W. 1890. Über den Reifungsprozeß des Selachiereies. Zeits. wiss. Zool., 50: 428.
- Makino, S. 1934a. The chromosomes of the sticklebacks, *Pungitius tymensis* (NIKOLSKY) and *P. pungitius* (LINNAEUS). Cytologia, 5: 155.
- 1934b. Notes on the chromosomes of some fresh-water Teleosts. (Japanese). Jap. Journ. Genet., 9: 100.
- 1937. Notes on the chromosomes of some teleost fishes. Zool. Mag. (Japan), 49: 75.
- Matthey, R. 1931. Chromosomes de reptiles sauriens, ophidiens, cheloniens. L'évolution de la formule chromosomiale chez les sauriens. Rev. Suisse de Zool., 38: 117.
- 1933. Nouvelle contribution à l'étude des chromosomes chez les sauriens. Rev. Suisse de Zool., 40: 281.
- Moore, J. E. S. 1895. On the structural changes in the reproductive cells during the spermatogenesis of elasmobranchs. Quart. Journ. Micr. Sci., 38: 275.
- Nakamura, K. 1932. Studies on reptilian chromosomes III. Chromosomes of some geckos. Cytologia, 3: 156.
- 1935. Studies on reptilian chromosomes V. Chromosomes of *Japaraula swinhonis* (a lizard). Mem. Coll. Sci. Kyoto Imp. Univ., Ser. B, 10: 355.
- Oguma, K. and S. Makino 1932. A revised check-list of the chromosome number in Vertebrata. Journ. Genet., 26: 239.
- Prokofieva, A. 1934. On the chromosome morphology of certain Pisces. Cytologia, 5: 498.
- Ralston, E. M. 1934. A study of the chromosomes of *Xiphophorus*, *Platyopocilus* and *Xiphophorus-Platyopocilus* hybrids during spermatogenesis. Journ. Morph., 56: 423.
- Sato, S. 1935. A note on the pacific dog-fish (*Squalus suckleyi* GIRARD) in the coastal waters of Hokkaido, Japan. Journ. Fac. Sci. Hokkaido Imp. Univ. Ser. VI. Zool., 4: 127.
- Schreiner, A. und K. E. Schreiner 1907. Neue Studien über die Chromatinreifung der Geschlechtszellen II. Arch. Biol., 22: 419.
- Vaupel, J. 1929. The spermatogenesis of *Lebistes reticulatus*. Journ. Morph., 47: 555.
- Winge, Ö. 1922. A peculiar mode of inheritance and its cytological explanation. Journ. Genet., 12: 137.
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## Spindle Orientation and Spore Delimitation in *Gelasinospora tetrasperma*

By

B. O. Dodge<sup>1)</sup>

The side by side orientation of the spindles in conjugate division of the nuclei in basal cells of sori of the rusts, in clamp connections of basidiomycetes and in crosiers of ascomycetes serves a very useful purpose. In the basidium of the mushrooms the spindles of the second division are more commonly placed at right angles to the long axis of the basidium. If the spindles of a species were regularly crossed and the final position of the four spores actually represented relatively the original location of the four nuclei after the second division, then spindle orientation could be made use of in genetic studies. Levine (1913), Wakayama (1930) and others have pointed out, however, that the orientation of spindles, crossed or parallel, in a basidium is not necessarily fixed, therefore one must conclude that this feature could have little meaning. Quite otherwise is the situation in ascomycetes like *Neurospora*, *Gelasinospora*, *Pleuraea* and *Bombardia*.

In *Neurospora tetrasperma* each spore is normally provided at its origin with two nuclei which are opposite in their sex reactions. The mechanism by which this is brought about has been fully described and diagramed (Dodge, 1927; Colson, 1934). In all species of *Neurospora* so far studied, a fourth nuclear division follows after spore delimitation, so that in the 8-spored species each spore has two nuclei, and in the 4-spored species each spore contains four nuclei at maturity.

Those who have studied *Pleuraea anserina* have not shown nuclear divisions in the ascus so that we are in the dark as to spindle orientation and the mechanism of delimitation. All agree that there are only two nuclei in the bisexual spore at maturity. In *Gelasinospora tetrasperma* where mature normal spores are 4-nucleate it might be assumed that the fourth nuclear division occurs after the spores are cut out, but Dowding (1933) did not follow up this point. She shows no 2-nucleate spores in 4-spored asci. We now have several facultatively heterothallic 4-spored species of ascomycetes. The

1) This study was based on material fixed by Miss Iris Campbell from her cultures during various hours of the day. She also assisted in the preparation of many of the slides. The writer is greatly indebted to her for this work. She is publishing an account of her culture work on single-spore isolates in another connection.

details in spindle orientation are known only for *Neurospora tetrasperma*. One cannot assume on that basis that orientation and spore delimitation are the same in all such species. The results of a cytological study of the ascus of *Gelasinospora* are presented at this time.

### Spindle orientation in *Gelasinospora tetrasperma*

Our material for a study of nuclear behavior in the ascus was obtained from matings of our strains Gel. 1 and Gel. 5. Whether or not the hours at which fixation was made had anything at all to do with it, we found that fixations between 7:30 and 11:30 P.M. gave the most division figures. Sections 7–10  $\mu$  thick are to be preferred where one wants to see the nuclei and spindles, and is not particularly concerned with delicate staining to show chromosome numbers.

### First nuclear division

From a study of 19 different first division figures it appears that the spindle is always intranuclear up to metaphase (Figs. 1–3). Centrosomes with radiating rays are plainly visible. An occasional spindle may be somewhat oblique at first (Fig. 2), but as a rule the elongated spindle is parallel to the long axis of the ascus (Fig. 6). Wilcox (1928) brought together in a table some of the data bearing on spindle orientation in ascomycetes. Her studies of *Neurospora sitophila* showed that, as is more commonly the case with long narrow asci, the first spindle is placed parallel to the long axis of the ascus.

We have seen only a few asci of *Gelasinospora* in the 2-nucleate stage. At first (Fig. 7) the nuclei are separated by a little more than the length of the spindle at its longest. Later they move farther apart and they must immediately begin the second division.

### Second nuclear division

Early stages of second division show rather spherical nuclei with distinct spindles (Fig. 8). Later the nuclei elongate with the spindles (Figs. 9, 10). Because asci are often cut obliquely and thus show only one of the two dividing nuclei, one must study adjacent sections to find the second nucleus. There is never any doubt as to which division, first or second, one sees, because of the difference in size of the nuclei and the length of the extended spindles. Spindles of the second division are also parallel to the long axis of the ascus. Although we have 32 different asci showing the second nuclear division and in all cases one spindle is well above the other and separated from it by a considerable distance, we are still uncertain as to just how it is that

the four nuclei reach their final positions. This is an important point because the relationship of the members of the pairs that go to opposite ends of the ascus determines the relationship of the two nuclei finally included in each ascospore. We first see four nuclei (Fig. 11) scattered along the more central part of the ascus. They are not very large and they are only slightly elongated.

#### The 4-nucleate stage

In all we have sections showing 71 different asci in the 4-nucleate stage. The final picture is always much the same. The nuclei are beaked, often showing short horn-like appendages, thus resembling nuclei of *Neurospora tetrasperma* at this stage. These appendages, whatever may be their nature, are not as noticeable as they are in *N. sitophila* (Wilcox, 1928). With the characteristic orientation of the four nuclei and their spindles in the third division in the ascus of *N. tetrasperma*, we once thought that it would not make any difference ordinarily whether the sex factors are segregated at the first or at the second division, in any case, each spore would be given two nuclei of opposite sex. The spindles of the second division, however, often lie one well above the other and parallel to the long axis of the ascus (Dodge, 1927) just as they are regularly in *Gelasinospora tetrasperma*. In all of the asci of *N. tetrasperma* observed in the 4-nucleate stage the nuclei were placed at the angles of a sort of parallelogram figure. It was assumed, therefore, that where second division spindles were placed one above the other, as just noted, there must follow a readjustment of the nuclei, much as though both spindles elongated extensively. Such a readjustment would imply a sex attraction of some kind. Sister nuclei of *N. sitophila* following second division show very distinct horned beaks. These beaks point away from each other showing a very characteristic nuclear orientation. In such long narrow asci it would be difficult for nuclei of opposite sex to pair up. With *N. tetrasperma* it is always an easy matter. If, however, segregation of the sex factors occurred at the second division, the two nuclei forming the pair could have come from the same mother nucleus and no extensive readjustment would be necessary to insure the inclusion of two nuclei of opposite sex in a spore.

It should be noted here also that occasionally one finds a spore, normal in size, but which proves to be unisexual though it must have originally contained two nuclei. With segregation at the first division followed by the "one above the other" orientation of the second division spindles, the mechanics would be right for cutting out binucleate unisexual spores, provided no readjustment of nuclei occurred (Dodge,

1936). Culture work to determine more accurately the percentage of unisexual spores that are cut out in asci with only four spores is being carried out with *Neurospora*, *Gelasinospora* and *Pleuraea*.

In our *Gelasinospora* even with the spindles oriented one well above the other, the four resulting nuclei come to rest, two at each end, rather far back at the limits of the region in the ascus which, because of its granular deeply staining material, may be referred to as the sporeplasm in contrast to the more vacuolate end portions (Fig. 13). In this ascus the nuclei are at the extreme limits. They do not usually move quite so far apart (Fig. 12). We have only two or three cases out of a total of 71 where the four nuclei occupy a more central position. These few cases probably represent early stages, or the picture before nuclear migration is completed. The fact that in all of the 77 cases of third division found the pairs of dividing nuclei lie at opposite ends of the ascus, is further evidence in favor of the assumption of a reorientation of the four nuclei following second division.

As found in their final resting position two at each end of the ascus, the nuclei are elongated and somewhat beaked. Occasionally one sees very plainly the horn-like deeply staining appendages like those of *Neurospora* noted previously. An interesting difference in the two species is that in *N. tetrasperma* the beaks of the upper pair of nuclei point upward and those of the lower pair point downward, and thus away from each other, while in *Gelasinospora* the beaks of the upper pair normally point downward and those of the lower pair point upward, and therefore toward each other. As will be noted later we found in our *Gelasinospora* only two asci in which the nuclear beaks pointed sidewise.

The nucleolus is always visible at the 4-nucleate stage, but other nuclear features are not well differentiated in our preparations. That part of the ascus at this stage containing the sporeplasm is somewhat enlarged as it is in *Neurospora*, but in *Gelasinospora* the pairs of nuclei are farther apart. The finely granular sporeplasm occupying the enlarged more central portions of the ascus is clearly differentiated from the more vacuolate less stainable epiplasm at the ends. In both species the 4-nucleate stage persists a long time, judging by the frequency with which this stage is found.

### Third nuclear division

In view of the very characteristic beaks of *Neurospora* it would be highly desirable to find early stages in third division to see what becomes of the horn-like deeply staining more or less forked beak



ends. The long ends of the fully extended spindles of the third division would suggest that they are the original nuclear appendages, and that they had simply pulled apart, one going one way and the other in the opposite direction across the ascus. Judging by what we see in *Gelasinospora* this is probably not the correct interpretation of those structures found at the ends of third division spindles in *Neurospora*.

We were fortunate in our studies of *Gelasinospora* to find four or five rather clear cut cases of early third division. No evidence indicating that the appendages of the nuclei represent dividing forked central bodies was found. In three cases the two nuclei had elongated considerably and showed delicate intranuclear spindles with chromosomes, centrosomes and rays. The pictures presented (Figs. 19-21) certainly suggest conjugate division and one can readily see how the spindles must necessarily be oriented at their final elongation. Some asci showed early third division with spindles somewhat crossed (Fig. 21). In all we have 77 different asci in which, with two exceptions to be noted later, the four nuclei can all be seen undergoing division. Usually the older spindles are deeply stained as though the fibers were massed. Aggregations of stainable granules appear at the spindle ends. One cannot see the reorganizing nuclei plainly until spore delimitation is under way. The orientation of the four spindles is certainly one of the most striking features in the whole life history of this interesting species. In ten cases the spindles in both pairs were more or less crossed (Fig. 15). In 37 asci the spindles in both pairs were parallel (Fig. 17) while in 30 asci the spindles were crossed in one pair and parallel in the other (Fig. 16). Probably in few cases were the spindles strictly parallel. One could see by focusing that, although the spindles appear parallel, they do not both lie in the same focus plane. A side view might show them as crossed.

As noted previously, we found one ascus in which the two nuclei at each end lay crosswise of the ascus (Fig. 22). One nucleus of each pair had already begun to divide showing short and very clear cut spindles in each case. These spindles are placed straight across the ascus. By focusing down one can see in the preparation the other missing nucleus of each pair. These two were still in the resting stage and showed the beak-end perfectly. In the illustration the nuclei at the lower focus are both drawn at the side. Their beaks point in the same direction.

In another ascus (Fig. 18) the nuclei of the upper pair were much swollen and were perhaps just beginning spindle formation. No doubt their spindles would have naturally extended down the ascus so that they would have appeared parallel. Both nuclei at the

lower end of this ascus had already divided, and the ends of the spindles were just breaking through the old nuclear membranes which had stretched out across the ascus. Chromosomes show rather plainly. This ascus is very narrow for one at this stage and it may be that some abnormal condition accounts for the position of these two spindles as well as the position and the condition of the two upper nuclei. It is not uncommon to find some one nucleus lagging behind the others a little as they undergo division, but these are the only two asci out of 77, counting early and late third divisions, in which two nuclei are so far behind the other two. In both cases the spindles present are at right angles to the long ascus of the axis.

### Ascospore delimitation

In *Neurospora tetrasperma* spindles of the third division disappear rather early and one then sees the pairs of nuclei attached to the ascus wall by their curved beaks. After the spores have been fully cut out and their nuclei have begun to move back or away from the wall, the old deeply staining beak-ends can still be seen near the wall. In *Gelasinospora*, however, spore delimitation is usually well under way before one can make out the nuclei very clearly, and the spindles persist so long that they must be cut across by the astral ray system (Figs. 23-25). In some asci at this stage the pairs of nuclei in a spore seem to have a characteristic orientation as they are attached to the spindle ends. After the spindles have disappeared one sees the two nuclei very distinctly with their long slender beak-ends from which a system of rays extends outward making a sort of sunburst effect (Figs. 26-28). The rays do not all proceed from a small centrosome. They also extend out from points well below the tip of the beak.

In some asci the pairs of nuclei in all four spores point downward (Fig. 28), while in other asci they all point upward at this stage (Fig. 27). Other asci show some pairs pointing down and others up (Fig. 26). The particular orientations of the pairs of nuclei probably are the same as they were at the time the nuclei were first reorganized and when they were still attached to the old spindles in early stages of spore formation (Figs. 23-25). The orientation of nuclear beaks in young spores can be of little significance as related to later developments.

The nuclei now break away from their beak ends and take on an elliptical form often still showing an orientation, their long axes more or less parallel to the long axis of the spore (Fig. 29). Asci showing spores with only two nuclei are not uncommon. We have catalogued about twenty examples but one could no doubt find several

more in our preparations. At this stage the spore content is rather finely granular and stains characteristically. The spores elongate somewhat and crowd upward to the ascus tip which has long been marked by the pore ring.

#### Fourth nuclear division

The fourth nuclear division in the ascus usually occurs simultaneously in all four spores (Fig. 30). One could not distinguish *Neurospora* from *Gelasinospora* at this stage. The spindles have no particular orientation although one occasionally finds parallel spindles in all four spores. Spindles are sometimes curved sharply, especially if they extend across the spore (Fig. 31).

Mature spores are normally 4-nucleate (Fig. 32) as has been shown previously by Dowding (1933). We have not studied delimitation either of the giant spores which must have several nuclei at their origin, or of the dwarf spores with only one nucleus.

Page (1936) reporting on *Podospora minuta*, a 4-spored species, says that mature spores are binucleate and that mycelia from dwarf, 1-nucleate, spores fall into two groups as to their sex reactions, "complementary" groups as she calls them. Spermatia present do not prove to have a function in fertilization. Page claims that there is an 8-spored form of this same species and in that form the mycelia which must come from 1-nucleate spores are totipotent. One is again left somewhat in doubt as to the identity of the 4-spored species which she studied. Dowding (1933) found just such a situation in *Gelasinospora*, but rightly referred to the two forms as distinct species.

On evidence that seems adequate, Dowding thinks that Page's (1933) "*Sordaria fimicola*" is really *Gelasinospora tetrasperma*. It certainly could not have been *Sordaria fimicola*. The mature spores of Page's *Sordaria* have four nuclei, showing that there must occur a fourth nuclear division before the spores are discharged, a feature characteristic of *Gelasinospora* and *Neurospora* and not of the 4-spored species of *Pleuroge* and *Podospora* so far studied.

#### Chromosome numbers

Many of the division figures in all four nuclear divisions show small chromosomes rather clearly in our preparations. We have made no attempt to count chromosomes at any stage, this task being left for another study which must also deal more fully with nuclear reorganization and the astral ray system involved in ascospore delimitation.

### Summary and conclusion

The nuclear spindles of the first and second divisions in asci of *Gelasinospora tetrasperma* are regularly placed parallel to the long axis of the ascus. The asci are at this time rather long and slender. At the 2-nucleate stage the nuclei come to lie one well above the other. In no case were the two spindles found parallel as though in conjugate division as is frequently the case in *Neurospora tetrasperma*.

At the close of the second division, the ascus contents become differentiated into two regions, the one containing finely granular material occupying the larger portion of the ascus. This represents that part of the ascus from which the ascospores will be delimited. It usually stains rather deeply as compared to the ends of the ascus where the contents are more vacuolate. The central part of the ascus is thicker than are the end portions.

The four reorganized nuclei move back near the end limits of the sporeplasm, one pair at each end. The nuclei are characteristically beaked and are normally oriented so that the beaks of the two pairs point toward each other. Rarely do the beaks point sidewise.

The elongated nuclei determine by their side by side orientation the first positions of the third division spindles. Since the asci are rather narrow as compared to the lengths of the fully extended spindles, the spindles are naturally forced to turn somewhat lengthwise in order to have room in which to elongate fully. In their final positions the spindles of a pair are either crossed, or they are parallel to the long ascus of the axis. When fully extended they never lie across the ascus as they do rather regularly in the ascus of *Neurospora tetrasperma*, where that part of the ascus containing the sporeplasm is much enlarged at this stage, and the spindles can lie directly across the ascus if necessary. They often seem to lie obliquely, however.

In *Gelasinospora* at the third division the spindles may be crossed in both pairs, or parallel in both pairs, or the spindles of one pair may be crossed while those of the other pair are parallel.

Third division spindles persist until spore delimitation is well under way, this being a very characteristic and striking feature.

Normally two elongated pear-shaped nuclei take part in cutting out each spore. As soon as the spore is well delimited the spindles connecting the pair of nuclei in adjacent spores disappear showing the nuclei now very clearly. They have long slender beaks capped by a sunburst of rays. If one did not see that the spore was already

delimited he might take this nuclear picture to represent the beginning of spore delimitation.

The nuclei break away from their beaks just as they do in *Neurospora*, round up somewhat and become more or less elliptical before going into the fourth division.

Mature ascospores in 4-spored asci have four nuclei each as previously reported by Dowding.

Considering the orientation of the spindles of the second division we must assume that there follows an extensive readjustment in the positions of the reorganized nuclei in order that they may become placed in pairs practically at the ends of a rather long ascus. There must be something comparable to a sexual attraction between the two nuclei to account for such a characteristic orientation. In such case it would not make any difference whether segregation of the sex factors occurred at the first division or at the second division, except in the latter case the reorganized nuclei would not have to travel as far. If the two nuclei at one end of the ascus are always derived from the same mother nucleus then segregation of the sex factors must commonly occur at the second division, which is highly improbable. What is needed now is more definite knowledge of the percentage of cases where binucleate unisexual spores are delimited. A further study to determine what happens immediately following second division would serve to explain how the final orientation of the four nuclei is brought about.

The New York Botanical Garden.

#### Literature cited

- Colson, B. 1934. The cytology and morphology of *Neurospora tetrasperma* [Shear &] Dodge. *Ann. Bot.* 48: 211-214.
- Dodge, B. O. 1927. Nuclear phenomena associated with heterothallism and homothallism in the Ascomycete *Neurospora*. *Jour. Agr. Res.* 35: 289-305. pl. 1-3. Fig. 1-5.
- Dowding, E. S. 1931. The sexuality of the normal, giant, and dwarf spores of *Pleurage anserina* (Ces.) Kuntze. *Ann. Bot.* 45: 1-13, pl. 1.
- 1933. *Gelasinospora*, a new genus of Pyrenomycetes with pitted spores. *Canadian Jour. Res.* 9: 294-305. pl. 1-3, fig. 1-9.
- Levine, M. 1913. Studies in the cytology of the Hymenomycetes, especially the Boleti. *Bull. Torrey Club* 40: 137-181, pl. 4-8.
- Page, W. M. 1933. A contribution to the life history of *Sordaria fimicola* (four-spored) with special reference to the abnormal spores. *Trans. British Myc. Soc.* 17: 296-301.
- 1936. Note on abnormal spores in *Podospora minuta*. *Trans. British Myc. Soc.* 20: 186-189.
- Wakayama, K. 1930. Contributions to the cytology of fungi. I. Chromosome numbers in Agaricaceae. *Cytologia* 1: 369-388.
- Wilcox, M. 1928. The sexuality and arrangement of the spores in the ascus of *Neurospora sitophila*. *Mycologia* 20: 3-17. pl. 2. fig. 1, 2.

## Explanation of plates

## Plate 32

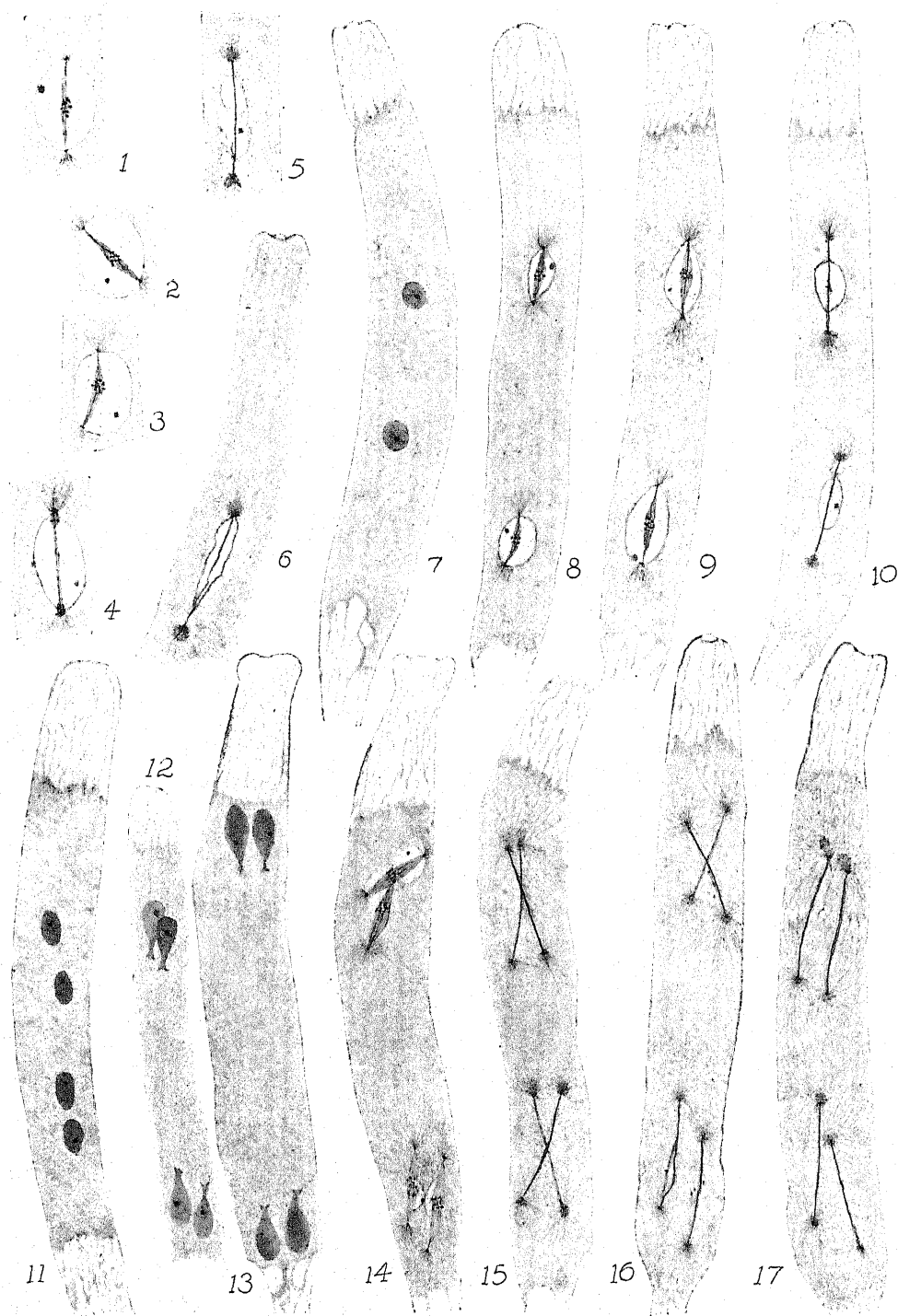
The figures are all drawn to the same scale

*Gelasinospora tetrasperma*

- Figs. 1-6. Various stages in the division of the primary nucleus in the ascus. The extended spindle lies parallel to the long axis of the ascus. The oblique spindle in one nucleus would naturally swing around in order to have space in which to elongate.
- Figs. 7-10. Finely granular central region differentiated as sporeplasm. Second nuclear division. The two extended spindles lie one well above the other so that as the four daughter nuclei are reorganized they must first lie more or less in a row as shown in 11.
- Fig. 11. The four daughter nuclei probably before readjustment in their positions has taken place.
- Fig. 12. Part of an ascus showing characteristic orientation of the four nuclei in two pairs at opposite ends of the ascus. The nuclei are beaked and sometimes show horn-like appendages.
- Fig. 13. This shows the pairs of nuclei at the extreme limits of the sporeplasm. They do not usually move so far apart. That part of the ascus containing the more granular and more deeply staining sporeplasm is somewhat swollen as compared to the ends containing the more vacuolate epiplasm.
- Fig. 14. The two pairs of nuclei are undergoing simultaneous conjugate division. See plate 2, figs. 19-21 for earlier stages in the third division.
- Fig. 15. The fully extended spindles of the third division, the spindles of both pairs are crossed. Reorganization of the daughter nuclei not yet far along.
- Figs. 16, 17. Other characteristic orientations of the pairs of spindles in third division. Nuclei are usually difficult to make out at such stages, probably not yet reorganized.

## Plate 33

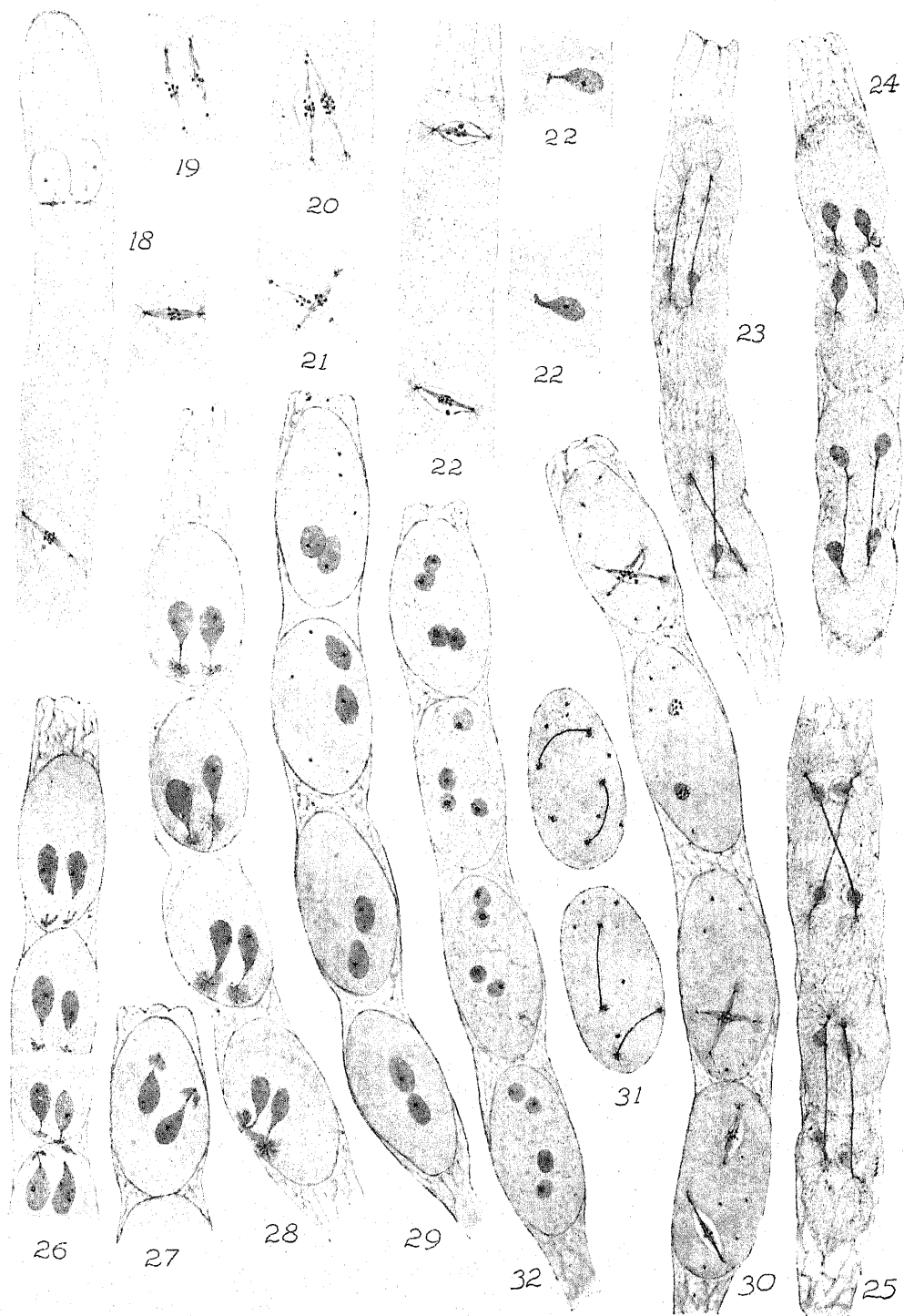
- Fig. 18. Rather narrow and perhaps abnormal ascus in which the nuclei of the upper pair are much swollen. Spindle formation may just be beginning, or it may be that these two nuclei are undergoing degeneration. The nuclei of the lower pair are dividing normally however. The orientation of their spindles across the ascus is exceptional. The nuclei may have extended across the ascus originally as was the case with the nuclei of the ascus shown in Fig. 22. The nucleus at the right was found in the next section, and is the mate of the one shown below in the ascus.
- Figs. 19-21. Pairs of dividing nuclei from different asci. Chromosomes show rather distinctly on the spindles.
- Fig. 22. The nuclei in both pairs must have extended across the ascus originally. Only one nucleus of each pair has divided. The other nucleus in each case (shown at the side) could be seen very clearly in the same section by focusing down farther. Their beaks extend in the same direction. This is the only case seen where both pairs of nuclei extend across the ascus.
- Figs. 23-25. Stages in spore delimitation; 23, a rather narrow ascus at an early stage in delimitation where the nuclei are not at all plainly visible; 24, 25, later stages in spore delimitation. The pairs of nuclei are now plainly visible and they are still attached to the old spindles. In Fig. 24 at the top, however, the spindles have disappeared. The beaks of all of the nuclei point downward and still show radiating rays. The persistence of the old spindles of the third division until after the spores are well delimited is rather unique, to say the least.



Dodge: Spindle Orientation and Spore Delimitation in *Gelasinospora tetrasperma*







Dodge: Spindle Orientation and Spore Delimitation in *Gelasinospora tetrasperma*



1000

(A)

7. 1000

- Fig. 26. Orientation of nuclei in the four spores from the same ascus. The nuclei in the lower spore are the only ones having their beaks pointing upward. (Third and fourth spores shown only in part.)
- Fig. 27. One of the four spores from an ascus where the beaks of all eight nuclei pointed upward.
- Fig. 28. Beautiful orientation of the four pairs of nuclei in the spores in an ascus. Here the beaks all point downward.
- Fig. 29. All of the nuclei have rounded up somewhat and are now ready for the fourth nuclear division. The cytoplasm in the spores is finely granular at this stage and stains rather deeply.
- Fig. 30. The spores are now fully elongated. The eight nuclei are all dividing. The spindles of the fourth division have no characteristic orientation. At this stage the spores usually contain a few small bodies that take the stain deeply.
- Fig. 31. Two spores showing a somewhat later stage in fourth division. The old spindles are curved following the contour of the spores.
- Fig. 32. The 16-nucleate stage, four nuclei in each spore. No further nuclear divisions will occur until germination begins. The cytoplasm is more vacuolate at this stage.
-

## Zur Kenntnis des Erbverhaltens einer gynodiözischen Pflanze, *Petasites japonicus* Miq.

Von  
S. Ikeno

(1)

Die Vererbungsverhältnisse der gynodiözischen Pflanzen wurden bisher nur selten studiert. Soweit mir bekannt ist, haben wir einerseits die alten Untersuchungen von DARWIN (1877) und andererseits die neueren ausführlichen von CORRENS (1904, 1905 usw.) vor uns. Nach den Resultaten meiner seit 1931 vorgenommenen Studien ist *Petasites japonicus* in die Klasse der gynodiözischen Pflanzen einzureihen, und dabei habe ich schon früher zweimal kurz darüber berichtet (1935 a, b). Wie man daraus sehen kann, weicht ihr eigentümliches Erbverhalten etwas von dem der bisher studierten gynodiözischen Pflanzen ab, weshalb *Petasites japonicus* wohl als ein neuer Typ dieser Klasse der Pflanzen aufzufassen ist. In der vorliegenden Mitteilung möchte ich dabei alle meine Experimentalresultate eingehender als früher schildern und noch einige neue Angaben hinzufügen.

*Petasites japonicus* MIQ. wird bei uns nicht nur sehr allgemein kultiviert, sondern auch im wilden Zustande weit verbreitet gefunden. Meine unten geschilderten Untersuchungen beziehen sich, 1. auf die Nachkommenschaft, welche aus den ursprünglich im Mai 1931 im Bergregion Hakone an den dort wildwachsenden Pflanzen gesammelten Achänen hervorgegangen sind (unten als *typica* bezeichnet) und 2. auf diejenige aus der Kreuzung *typica* × Kulturvarietät *purpurascens* MAKINO, welche letztere seit Jahren im botanischen Institut zu Komaba in

Kultur war. Diese Varietät ist dadurch ausgezeichnet, daß ihre verschiedenen Organe mehr oder minder stark purpurn gefärbt sind: Blattspreite an ihrer unteren Fläche, besonders stark im Frühling; Nerven an derselben ziemlich stark; Blattstiel; große Niederblätter, welche den ganzen Blütenstand im jungen Zustande bedecken, an ihrer inneren Fläche prächtig schön; Involukrallblätter jedes Blütenköpfchens an ihrer inneren Fläche; Staubfäden usw.. Beobachtet man den Querschnitt des Blattstiels, so wird man sehen, daß nicht nur alle Oberhautzellen, sondern auch einige unter denselben gelegene

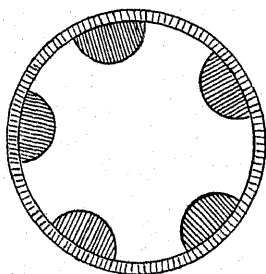


Fig. 1. Schematische Darstellung eines Querschnittes des Blattstiels von var. *purpurascens*. Anthozy-anhaltiger Teil schraffiert.

kleine keilförmige parenchymatische Zellgruppen anthozyanhaltig sind (Fig. 1). Weiter, in der Blattspreite ist das Anthozyan in den Oberhautzellen der unteren Fläche und einigen unter und längs derselben unterbrochen verlaufenden langschmalen parenchymatischen Zellgruppen enthalten.

## (2)

Eine reichliche Menge von 1931 gesammelten Achänen von *typica* wurde bald nach der Ernte gesät. Die Keimung derselben war sehr schlecht, und nur wenige Keimlinge sind daraus entstanden, von denen nur 8 Individuen leben blieben. 1932 sind davon 6 zum Blühen gekommen, während Nr. 7 erst später Blüten getragen hat und Nr. 6 überhaupt noch nicht blühte (s. Tabelle 1).

Tabelle 1

Jahr	Individuen-Nr.							
	1	2	3	4	5	6	7	8
1932	♂	♀	♂	♀	♀			♀
1933		♀	♂					
1934							♂	

Die Zahl der Blütenstände pro Individuum ist klein, gewöhnlich bloß ein, höchstens zwei, wenn die Anzahl der in jedem Blütenstand enthaltenen Blütenköpfchen ziemlich groß ist<sup>1)</sup>. Aus der Tabelle 1 kann man sehen, daß einige Individuen, welche schon in einem Jahre geblüht haben, im nächsten blütenlos geblieben sind (Nr. 1, 4, 5, 8).

Die wichtige Tatsache, welche wir aus der obigen Tabelle entnehmen können, besteht darin, daß wir bei *Petasites japonicus* mit einer gynodiözischen Pflanze zu tun haben, indem dabei zwei Sorten Individuen, nämlich zwittrige und weibliche, vertreten sind.

## (3)

Vergleichen wir nun beide oben bezeichnete Sorten Individuen miteinander. Die ganze Statur des Zwitter ist etwas größer als die des Weibchens, und dementsprechend ist der ganze Blütenstand, jedes Blütenköpfchen und jede einzelne Blüte größer bei dem ersteren als bei dem letzteren. Die Kleinheit jeder weiblichen Blüte im Vergleich zu der zwittrigen ist so beträchtlich, daß trotz der geringeren Größe des weiblichen Köpfchens die Zahl der enthaltenen Blüten viel größer bei dem weiblichen als bei dem zwittrigen ist. Um ein Beispiel zu nennen: bei ♀ Köpfchen 44 ♀ Blüten und bei ♂ 88 ♀ (+ 3 ♂). Dabei ist zu bemerken, daß jedes zwittrige Köpfchen ausschließlich aus zwittrigen Blüten zusammengesetzt ist, während sich in dem weiblichen außer den weiblichen Blüten nicht selten einige kleine rudimentäre zwittrige mit

1) Wegen Raumersparnis wurden alle Stöcke bei ziemlich schmaler Drillweite gepflanzt; wäre sie weiter gemacht worden, könnte jeder noch besser angewachsen und mehrere Blütenstände gebildet haben.

leeren Antheren im Zentrum des Köpfchens befinden (z.B. 3 solche Blüten im soeben zitierten Beispiele).

In Fig. 2a ist eine weibliche und in Fig. 2b eine zwittrige Blüte dargestellt. Beides sind in gleichem Maße etwas vergrößerte photo-

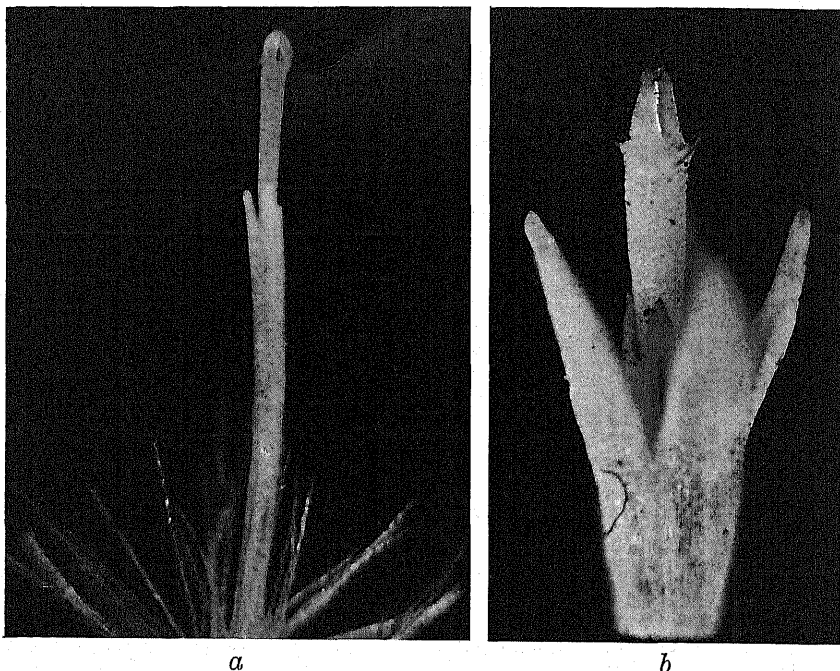


Fig. 2. Photographische Aufnahmen etwas vergrößert, und zwar im gleichen Maße. *a*, weibliche und *b*, zwittrige Blüte. *a* Griffel schmallang mit zwei Narben; Blütenkrone röhrenförmig und am oberen Ende zwei Lappen unter fünf sichtbar; Fruchtknoten nicht und Pappus teilweise sichtbar. *b* Griffel, Narben und oberer Teil der Blütenkrone sichtbar (drei Lappen sichtbar). Alle Organe in *b* sind beträchtlich dicker als die entsprechenden in *a*.

graphische Aufnahmen. Wenn man diese zwei Photographien miteinander vergleicht, wird man sofort zwischen beiden einen beträchtlichen GröÙeunterschied bemerken. Für weiteres sei auf die Figurenklärung verwiesen.

#### (4)

Um die Vererbungsverhältnisse von beiderlei Sorten Blüten kennen zu lernen habe ich 1932 die Bestäubung Nr. 2  $\times$  Nr. 1 sowie Nr. 8  $\times$  var.

Tabelle 2. (Nr. 2  $\times$  Nr. 1  $\sigma$ ) (1932)

Jahr	Individuen-Nr.						
	2-1	2-2	2-3	2-4	2-5	2-6	2-7
1933	+	+	+	+	+	+	+
1934	+	+	+	+	+	+	+
1935	+	+	+	+	+	+	+

*purpurascens*, und 1933 Nr. (2-4)  $\times$  (2-2) und (2-6)  $\times$  (2-5) vorgenommen. Die Resultate davon sind in der Tabelle 2-3 usw. enthalten.

Aus der Bestäubung Nr. (2-4)  $\times$  (2-2) sowie (2-6)  $\times$  (2-5) sind je 1 Zwitter bzw. 1 Weibchen entstanden.



Tabelle 3. (Nr. 8♀ × var. *purpurascens* ♂) (1932)

Jahr	Individuen-Nr.																
	8-1	8-2	8-3	8-4	8-5	8-6	8-7	8-8	8-9	8-10	8-11	8-12	8-13	8-14	8-15	8-16	8-17
1933	♂	♂	♀	♂	♀	♂	♂	♂	♂	♂	♂						
1934	♂	♂	♀	♂		♂	♂	♂	♂	♂	♂		♀				
1935	♂	♀	♀	♂		♂	♂	♂	♂	♂	♂	♂	♀	♂		♀	♀

Alle soeben erörterten Experimente haben uns gezeigt, daß 1. die weibliche Blüte fruchtbar ist, wenn sie mit dem Pollen des Zwitter bestäubt wird und daß 2. die zwitterige Blüte (beide *typica* und *purpurascens*) immer ganz unfruchtbar bleibt, sei es durch Selbst- oder Fremdbestäubung trotz des ziemlich gesunden Aussehens des Griffels und der Narben derselben (siehe Fig. 2b). Weil aber bei der zwitterigen das Pollen tauglich ist, wie oben hervorgehoben, ist sie physiologisch als männlich zu betrachten, und somit wäre in diesem Sinne *Petasites japonicus* vielleicht als eine diözische Pflanze zu betrachten. Die unmittelbare Ursache der Unfruchtbarkeit der zwitterigen Blüte ist nicht ohne weiteres klar. Ich habe einigemal ein gequetschtes Präparat der Griffel und Narben einer am vorhergehenden Tage reichlich bestäubten zwitterigen Blüte unter 0,1% Kottonblaulösung studiert, habe aber darin keinen Pollenschlauch finden können. Darum scheint es mir wahrscheinlich zu sein, daß auf den Griffel und Narben der zwitterigen Blüten überhaupt kein Pollen keimen kann. Diese Tatsache bedarf aber wohl noch weiterer Untersuchung, weil meine diesbetreffende Beobachtung nicht umfangreich genug ist.

## (5)

In den obigen Tabellen haben wir nicht selten solche Individuen angetroffen, welche in verschiedenen Jahren verschiedene Arten Blütenstände produziert haben (Tab. 2, Nr. 2-4, 2-6; Tab. 3, Nr. 8-2, 8-6, 8-7, 8-8). Die Frage ist nun, was ihre genotypische Natur sein wird. Dieses eigentümliche Phänomen könnte man vielleicht für eine Geschlechtsumwandlung nehmen; meiner Ansicht nach ist es aber eher der zufälligen Ausbildung der Blüten des entgegengesetzten Geschlechtes eines bestimmten Individuums vergleichbar, wie man häufig bei diözischen Pflanzen (z.B. *Salix*) beobachtet hat und oft in der Literatur erwähnt findet<sup>1)</sup>, wenn es auch in unserem Falle wegen der gleichartigen Zahl von

1) Um ein von mir beobachtetes konkretes Beispiel eines solchen Phänomens anzugeben, habe ich vor vielen Jahren an einem Stock von *Salix amygdalina* L. var. *nipponica* SCHNEID. mit einer großen Anzahl von weiblichen Kätzchen das Erscheinen einiger männlichen nachgewiesen. Die Bestäubung der ersteren durch das Pollen der letzteren hat zur Bildung einer reichlichen Menge von Früchten geführt—die sog. Geitongamie.

beiderlei Art Blütenständen pro Individuum (gewöhnlich nur ein oder zwei) sowie des Zeitunterschiedes ihres Entstehens an demselben etwas fremdartig erscheinen mag, im Vergleich z.B. zum typischen Beispiele von *Salix*, welches in der Fußnote der vorigen Seite erwähnt wird <sup>1)</sup>.

Es fragt sich, ob man die in Rede stehenden Individuen unter die weiblichen oder zwitterigen einreihen muß. Es wird jetzt im allgemeinen angenommen, daß das Zwittertum phylogenetisch den primären, und die Diözie einen daraus entstandenen sekundären Zustand darstellt. Auf Grund dieser Hypothese, welche mir ganz richtig zu sein scheint, fasse ich alle Pflanzen, welche zweierlei Art Blütenstände ausgebildet haben, genotypisch immer als die weiblichen auf, wobei zufälligerweise die zwitterigen Blütenstände entstanden sind. Das Vorhandensein weniger rudimentären zwitterigen Blüten im weiblichen Köpfchen, wie oben hervorgehoben, spricht für diese Auffassung.

	♂	♀
Tabelle 1	4	3
Tabelle 2	3	4
Tabelle 3	13	6
(2-6) × (2-2)		1
(2-6) × (2-5)	1	
Summe	21	14
Ganze Summe	= 35	
Erwartet	17,5	17,5

Wenn man unter dieser Voraussetzung die Zahl der auf Tab. 2-3 angegebenen Nachkommen beider Arten ausrechnet und dazu noch je 1 Individuum aus (2-4) × (2-2) und (2-6) × (2-5) hinzufügt, so hat man: (s. links!)

Führt man über die obigen Zahlen eine statistische Rechnung und zwar unter der Theorie, daß beiderlei Art Individuen an Zahl gleich sein müssen, ist die Abweichung aus der Erwartung ( $= \pm 3,5$ ) ein wenig größer als der Standardfehler ( $= \pm 2,9$ ). Darf man dann hier die theoretische Erwartung der Gleichheit von zwei Arten Nachkommen nicht annehmen? Ich glaube doch. Die soeben angedeutete kleine Abweichung dürfte meiner Ansicht nach hauptsächlich von der geringen Anzahl der Individuen kommen und wenn man daher noch zahlreichere zur Rechnung benutzen könnte, wäre höchst wahrscheinlich eine viel bessere Übereinstimmung mit der Theorie zu erwarten. Wenn es dann trotzdem noch nicht der Fall ist, müßte vielleicht irgend eine Ursache vorhanden sein, welche die in Rede stehende Gleichheit stört <sup>2)</sup>.

1) Wenn weibliche und zwitterige Blüten an ein und demselben Individuum vorkommen, spricht man dabei von einer gynomonözischen Pflanze. In diesem Sinne kann man wohl jede *Petasites*-Pflanze, welche in einem Jahre weibliche Blütenstände und in einem anderen zwitterige ausgebildet hat, als gynomonözisch bezeichnen. Diese Tatsache bei *Petasites* ähnelt etwas der, was CORRENS (1905) bei einer gynodiözischen Pflanze, *Satureia hortensis* beobachtet hatte, wobei seit einem gewissen Jahre beiderlei Arten Blüten immer an ein und demselben Individuum vorgekommen sind (d.h. gynomonözisch), wenn in diesem Falle beide zugleich an jedem Stock zu sehen waren, im Gegensatz zu *Petasites*.

2) Bekanntlich wird häufig angenommen, daß die Abweichung aus der Theorie, welche kleiner ist als dreimal  $\sigma$  (hier  $= 3 \times 2,9 = \pm 8,7$ ) innerhalb der theoretisch zu erlaubenden Grenze liegt.

Ob nun bei unseren Versuchen die soeben genannte theoretische Erwartung wirklich erfüllt ist oder nicht, man kann jedenfalls daraus wie folgt schließen. Das oben erörterte Entstehen von beiden Zwitter- und Weibchen-Nachkommen aus dem Weibchen ♀ × Zwitter ♂ muß durch eine Bastardierung zwischen einem hetero- und einem homozygotischen Individuum, oder anders ausgedrückt, durch eine Rückbastardierung hervorgebracht werden. Wir haben dabei zwei Fälle zu unterscheiden: 1. Weibchen hetero- und Zwitter homozygotisch, und 2. Weibchen homo- und Zwitter heterozygotisch. D.h.

$$1. Aa \text{ ♀} \times aa \text{ ♂} = Aa \text{ ♀} + aa \text{ ♀}$$

$$2. aa \text{ ♀} \times Aa \text{ ♂} = aa \text{ ♀} + Aa \text{ ♀}$$

wobei im 1. Falle die Weiblichkeit über das Zwittertum dominiert und im 2. es gerade umgekehrt ist. Die Frage, mit welcher von diesen zwei Alternativen wir es hier zu tun haben, kann entweder experimentell oder zytologisch gelöst werden. Experimentell kann man z.B. die wohlbekannte CORRENSsche Zertationsmethode anwenden; dabei muß eine reichliche Menge von Nachkommen zur Verfügung stehen, jedoch ist bei *Petasites* nicht nur das Keimungsprozent von Samen im allgemeinen sehr niedrig, sondern es gehen auch viele Keimlinge früh zu Grunde, und es wäre nicht sehr leicht, eine für den Zweck unserer Untersuchung genügende Menge von Nachkommen zu bekommen. Andererseits könnte man zytologisch untersuchen, ob sich in unserem Falle irgendwelche Geschlechtschromosomen nachweisen lassen, und wenn ja, was ihr Verhalten sein wird usw. Nach den im hiesigen Institute von K. SUZUKI gemachten vorläufigen Beobachtungen ist dabei nicht nur die Zahl von Chromosomen groß ( $2n =$  mehr als 80), sondern sie sind auch ziemlich lang, und es ist zweifelhaft, ob man die sich daraus ergebenden Schwierigkeiten der Beobachtung überwinden kann, um das ganze auf zytologischem Grunde festzustellen. Die soeben erörterten erheblichen Schwierigkeiten der Experimente und Beobachtungen haben uns bisher verhindert, solche vorzunehmen, und die Frage der Hetero- und Homozygotie der Weibchen und Zwitter bleibt daher leider hier noch unentschieden<sup>1)</sup>.

### (6)

Vergleicht man unseren Fall mit dem der von CORRENS (1904) genau untersuchten gynodiözischen Pflanze *Satureia hortensis*, nimmt man zwischen beiden einen beträchtlichen Unterschied des Erbverhaltens wahr. Bei *Satureia hortensis* nämlich sind sowohl Zwitter wie Weibchen fruchtbar, und jede Art bringt ausschließlich oder zumindest vorwiegend die

1) Es würde vielleicht auch von Interesse sein, das karyologische Verhalten unserer Pflanze und dasselbe von gewissen Zwischengeschlechtstypen, wie z.B. bei *Rumex acetosa* vergleichend zu untersuchen, um kennen zu lernen, ob zwischen beiden irgend eine Analogie bestehen mag.

eigene Art hervor (d.h. Weibchen Weibchen und Zwitter Zwitter). Wie oben hervorgehoben, ist bei *Petasites* das Weibchen allein fruchtbar, und der Zwitter dient bloß als Pollenlieferant, sodaß unsere Pflanze sich mehr der rein diözischen Pflanze nähert als der bisher studierten gynodiözischen. Wenn man die Gynodiözie als eine phylogenetische Übergangsstufe aus dem Zwittertum zu der reinen Diözie betrachtet, muß man annehmen, daß *Petasites* eine Stufe ist, welche phylogenetisch etwas weiter nach dem letzteren Zustand fortgeschritten ist als *Satureia hortensis*.

Weiter, unter allen bisher untersuchten gynodiözischen Pflanzen steht das Verhalten von *Petasites* einzig da, und sie ist somit als ein neuer Typ anzunehmen. Nun erwähnt DARWIN (1877 S. 298 ff.) bei *Thymus serpyllum* das Entstehen je einer Menge von beiden weiblichen und zwitterigen Nachkommen aus dessen Weibchen, und es wäre nicht unwahrscheinlich, daß *Thymus* zur gleichen Kategorie der gynodiözischen Pflanzen wie *Petasites* gehört. Diese Annahme kann jedoch natürlich nicht so ohne weiteres als sicher hingestellt werden.

(7)

S. 888 habe ich die Merkmale von var. *purpurascens* inbezug auf die purpurne Farbe ihrer verschiedenen Organe mitgeteilt. Von dieser Pflanze steht mir bloß der Zwitter zur Verfügung und ich habe noch niemals das Weibchen gesehen. Die Resultate der Kreuzung *typica* ♀ × *purpurascens* ♂ sind in der Tab. 3 hervorgehoben. Es ist wohl bekannt, daß bei bisher untersuchten Bastarden das farbige Merkmal über das nichtfarbige in den meisten Fällen dominiert, und zwar kann man schon im Keimlingstadium von F<sub>1</sub>-Generation das Dominieren des Farbmerkmals klar feststellen (z.B. Kotyledonen usw. gefärbt). Ich habe erwartet, daß man auch in unserem Fall das gleiche beobachten wird. Diese Erwartung wurde aber nicht erfüllt, weil alle Keimlinge und auch alle daraus entstandenen ausgewachsenen Pflanzen keine Spur der Purpurfarbe erkennen ließen.

Ein solches Verhalten der F<sub>1</sub>-Pflanzen würde eintreten, wenn das Farbmerkmal gegenüber dem ungefärbten rezessiv ist, z.B. durch die Mitwirkung des an dem farblosen Individuum vorhandenen Hemmungsfaktoren, und dann kann die Farbe z.B. durch die Rückbastardierung sichtbar gemacht werden, so in unserem Fall

*typica* = HH, *purpurascens* = hh (H = Hemmungsfaktor), und Rückbastardierung

$$\begin{array}{ccccccc}
 (typica \times purpurascens) \times purpurascens & = & typica + purpurascens \\
 H \times h & \times & h & = & Hh + hh
 \end{array}$$

Im Frühjahr 1935 habe ich einige Individuen (*typica* × *purpurascens*) (Tab. 3) durch das Pollen des ursprünglich zur Kreuzung benutzten

Law  
(A)  
7. März

*purpurascens*-Mutterstockes bestäubt, d.h. (*typica* × *purpurascens*) × *purpurascens* und eine reichliche Menge von Achänen bekommen. Die Keimung derselben war ziemlich gut. Es sind daraus ungefähr 50 Keimlinge entstanden, von denen 25 ausgewachsen sind. Es muß so- gleich erwähnt werden, daß keiner dieser Keimlinge oder ausgewachsenen Individuen irgendeine Spur der Purpurfarbe erkennen ließ. Somit war es mir sogar mittels des Rückbastardierungsverfahrens unmöglich, gefärbte Nachkommen zu bekommen. Was wird wohl die Ursache davon sein? Zur Erklärung dieses eigentümlichen Verhaltens möchte ich die folgende Hypothese vorschlagen: die Übertragung des anthozyanhaltigen Zytoplasmas an die Nachkommen findet ausschließlich durch die Eizelle statt. Wie wohl bekannt, wird nicht selten die Buntblät- terigkeit ausschließlich durch die Mutter an die Nachkommen übertragen und niemals durch den Vater. Diese Tatsache, sog. mütterliche Ver- erbung, wird bekanntlich gewöhnlich so aufgeklärt, daß bei der Befruch- tung der männliche Zellkern, welcher im nackten Zustande in die Eizelle eindringt, keine kranken Plastiden (BAUR 1909) oder krankes Zytoplasma (CORRENS, z.B. 1927, bes. S. 139 ff) ins Eiinnere mitnimmt und die „Ver- erbung“ der Buntkrankheit ausschließlich mittels der im Eizytoplasma vorhandenen kranken Plastiden oder des kranken Zytoplasmas erfolgt.

Bei *Petasites japonicus* var. *purpurascens* sowie ihren Bastarden könnte die „Vererbung“ der Purpurfarbe durch die Wirkung des antho- zyanhaltigen Eizytoplasmas erfolgen, ähnlich dem oben genannten Fall der Buntblättrigkeit<sup>1)</sup>. Auf Grunde dieser Annahme ist die Tatsache, daß bei unseren Versuchen, bei welchen *purpurascens* bloß als Pollen- lieferant benutzt werden konnte, keine „Vererbung“ der Purpurfarbe nachzuweisen war, kein Wunder. Die Übertragung der Farbe an die Nachkommen ausschließlich durch die gefärbten Mütter scheint mir in unserem Falle kaum zweifelhaft zu sein. Das ist jedoch erst durch Ex- perimente, wobei solche Mütter wirklich benutzt werden, sicherzustellen. Das Experiment ist mir augenblicklich unmöglich wegen Mangel an *purpurascens*-Weibchen<sup>2)</sup>.

(8)

Fassen wir schließlich alles oben gesagte kurz zusammen. *Petasites japonicus* ist, wie durch Experimente nachgewiesen ist, eine gynodiö- zische Pflanze, bei welcher zwei Arten Individuen, nämlich das Weibchen und der Zwitter zur Ausbildung kommen. Das erstere allein ist frucht-

1) Da es bei var. *purpurascens* natürlich keine anthozyantragende Plastiden gibt, kann hier von der Übertragung derselben keine Rede sein.

2) Einer brieflichen Mitteilung des Herrn Dr. T. MAKINO, dem besten Kenner der japanischen Pflanzen in der Gegenwart, ist diese purpurne Varietät bei uns sehr selten. Es ist daher kaum zu erwarten, daß ich ihren weiblichen Stock in näherer Zukunft bekommen kann.

bar und daraus kommen beiderlei Arten Nachkommen. Der letztere dagegen ist ganz unfruchtbar und bloß als Pollenlieferant bei der Befruchtung dienlich. Der letztere Vorgang ist als eine Rückbastardierung, Heteroweißchen ♀ × Homozwitter ♂, oder Homoweißchen ♀ × Heterozwitter ♂ aufzufassen, und dabei ähnelt das ganze Verhalten weit mehr dem der rein diözischen Pflanzen als dem der bisher untersuchten gynodiözischen, z.B. *Satureia hortensis*. Man kann wohl *Petasites* als eine Übergangsstufe aus dem Zwittertum zur Diözie, welche phylogenetisch einen Schritt weiter fortgeschritten ist als *Satureia*, annehmen.

Var. *purpurascens*, welche morphologisch ganz *typica* ähnlich ist, ist durch die purpurne Farbe ihrer verschiedenen Organe ausgezeichnet. Es ist höchst eigentümlich, daß die Kreuzung *typica* ♀ × *purpurascens* ♂ sowie (*typica* ♀ × *purpurascens* ♂) × *purpurascens* ♂ eine Nachkommenschaft liefern, welche keine Spur der purpurnen Farbe erkennen läßt. Diese experimentellen Resultate haben mich dazu geführt, die Hypothese vorzuschlagen, daß das anthozyanhaltige Zytoplasma ausschließlich durch die Eizelle an die Nachkommenschaft übertragen werden kann.

#### Schriftenverzeichnis

- Baur, E. (1904). Das Wesen und die Erblichkeitsverhältnisse der „Varietates albo-marginatae hort.“ von *Pelargonium zonale*. Zeitsch. ind. Abstamm. u. Vererb. lehre 1, S. 330.
- Correns, C. (1904). Experimentelle Untersuchungen über die Gynodiöcie. Ber. Deutsch. Bot. Ges. 22, S. 397.
- (1905). Weitere Untersuchungen über die Gynodiöcie. Ibid. 23, S. 452.
- (1927). Über nichtmendelnde Vererbung. Zeitsch. ind. Abstamm. u. Vererb. lehre Supplement-Bd. 1, S. 131.
- Darwin, C. (1877). Different forms of flowers.
- Ikeno, S. (1935a). Gynodiözie von *Petasites japonicus* (in japanisch). Syokubutu oyobi Dôbutu 3, S. 59.
- (1935b). Hérité de la gynodiöcie chez le *Petasites japonicus*. C. R. Acad. Sc. Paris 198, S. 1471.

#### Nachträge bei der Korrektur

Meine ganze *Petasites*-Kultur wurde Mitte 1935 in das neue Versuchsfeld nach Hongô verpflanzt, und im Frühling 1936 sind keine Pflanzen zur Ausbildung von Blütenständen angekommen. Im März 1937 jedoch konnte man dieselben bei einer Anzahl von Individuen beobachten und dabei möchte ich die folgende Tatsache erwähnen.

Wie oben erörtert, habe ich bis zum Jahre 1935 die Ausbildung von höchstens zwei Blütenständen pro Individuum nachweisen können, aber diesmal (Frühling 1937) habe ich bei einem Individuum (Nr. 2A-3) fünf und bei einem anderen (Nr. 2A-9) drei Blütenstände gesehen. Beim ersteren sind alle weiblich, aber beim letzteren sind unter dreien zwei zwitterig und einer weiblich. D.h. die Blütenstände von entgegengesetzter Natur sind zugleich an ein und demselben Individuum vorhanden. Noch bei einem anderen (Nr. 2A-7), wo nur ein Blütenstand zu sehen war, habe ich unter zahlreichen zwitterigen Köpfchen ein einziges weibliches wahrgenommen.

Law  
(A)  
7. 7. 37



## Karyological Studies on Some Species of *Lobelia*

By

Shun Okuno

Hokkaido Imperial University

Concerning the basic chromosome number in the genus *Lobelia*, two different views have hitherto been advanced; ARMAND ('12) stated that the haploid number of *L. Dortmanna*, *L. Erinus* and *L. Urens* each is 8, whilst DE VILMORIN and SIMONET ('27b) reported 7 haploid chromosomes in *L. cardinalis*, *L. cliffortiana*, *L. syphilitica* and *L. Urens*, and also 14 and 21 chromosomes in several varieties of *L. Erinus*. The present study has accordingly been initiated with a primary aim to ascertain whether the genus contains types of both 7 and 8 basic numbers, or if not, which view is correct.

The material was obtained from the experimental gardens of our Laboratories in which several species of *Lobelia* from various sources are cultivated, with the exception of *L. sessilifolia* which was collected by the writer at Numanohata, Hokkaido.

For determining the somatic chromosomes of the root-tips, the usual paraffine method was employed, the fixative being NAWASCHIN's chromo-acetic solution and the stain being iron-alum haematoxylin. For the meiotic chromosomes in the PMCs, iron-acetocarmine smears were made in addition to the paraffine section.

### Observations

#### i) Chromosome numbers and morphology

The chromosome numbers in the seven species of *Lobelia* employed were determined as follows:

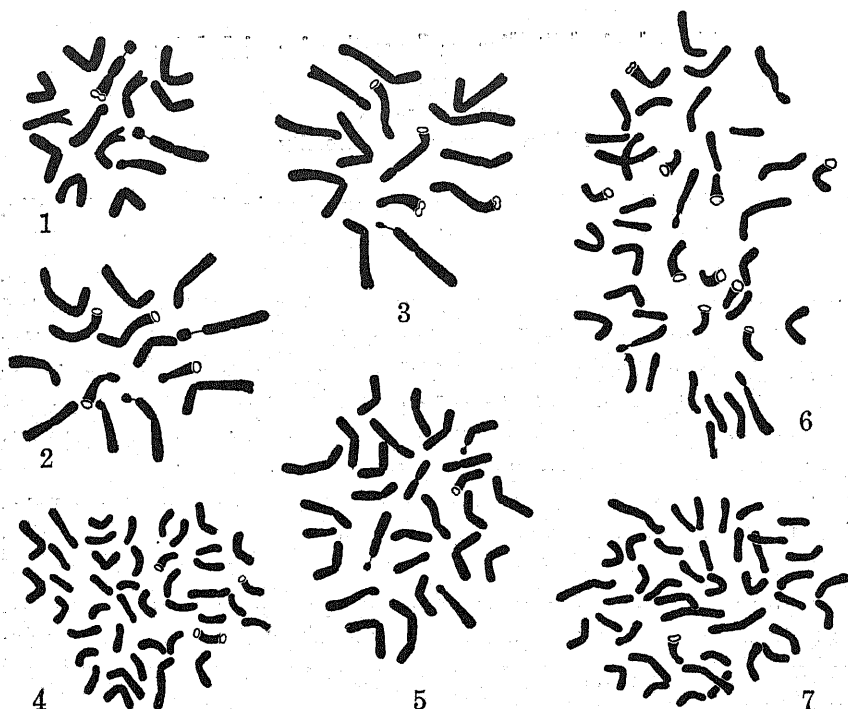
Species	Meiotic	Somatic
<i>L. inflata</i> L.	7 <sub>II</sub> (Fig. 10)	14 (Fig. 1)
<i>L. dresidensis</i> *	7 <sub>II</sub> (Fig. 11)	14 (Fig. 3)
<i>L. syphilitica</i> L.	7 <sub>II</sub> (Fig. 9)	14 (Fig. 2)
<i>L. sessilifolia</i> LAMB.	14 <sub>II</sub> (Fig. 13)	28 (Fig. 5)
<i>L. Erinus</i> L. Plant No. 1.	21 <sub>II</sub> (Fig. 16)	42 (Fig. 7)
„ „ Plant No. 2.	20 <sub>II</sub> +2 <sub>I</sub> (Figs. 14, 15)	—
<i>L. triquetra</i> L. Plant No. 1.	—	42 (Fig. 6)
„ „ Plant No. 2.	7 <sub>II</sub> (Fig. 17)	—
<i>L. Richardsonii</i> HORT.	—	42 (Fig. 4)

\* The author name of this species could not be traced.

As will be clear from the above list, the genus *Lobelia* consists of a polyploid series with the basic number of 7, i.e., a series of 7—



14-21. The present results are in agreement with the observation by DE VILMORIN and SIMONET.



Figs. 1-7. Somatic complements in 7 species of *Lobelia*. 1. *L. inflata* ( $2n = 14$ ). 2. *L. syphilitica* ( $2n = 14$ ). 3. *L. dresidensis* ( $2n = 14$ ). 4. *L. Richardsonii* ( $2n = 42$ ). 5. *L. sessilifolia* ( $2n = 28$ ). 6. *L. triquetra* ( $2n = 42$ ). 7. *L. Erinus* ( $2n = 42$ ).  $\times 5000 \times 2/3$ .

So far as it concerns the present study, the meiotic divisions are normal in general, though in one plant of *L. Erinus* a few univalents were often met with.

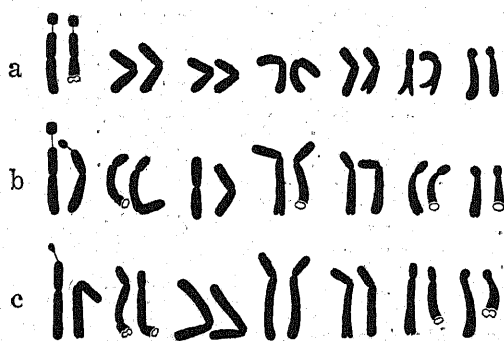
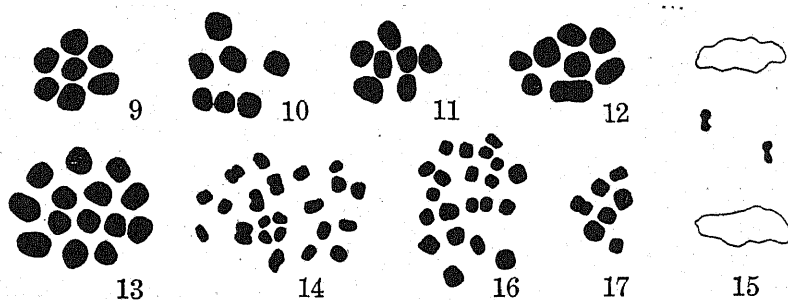


Fig. 8. The comparison of chromosome-types in *L. inflata* (a), *L. syphilitica* (b), and *L. dresidensis* (c).  $\times 5000 \times 2/3$ .

The comparison of idiograms in three diploid species, *L. inflata* (Fig. 8a), *L. syphilitica* (Fig. 8b) and *L. dresidensis* (Fig. 8c) reveals certain considerable differences amongst them, namely, (i) the chromosomes of *L. syphilitica* and *L. dresidensis* are fairly longer than those of *L. inflata*, (ii) there is one pair of

two constricted chromosomes in each of *L. syphilitica* and *L. dresidensis*, while *L. inflata* lacks the corresponding type, and (iii) in *L. inflata* there is one pair of satellited chromosomes, the satellites being of similar size; in *L. syphilitica* the corresponding pair is heteromorphic with respect to the size of the satellites; but in *L. dresidensis* one of the pair is deprived of the satellite. Such a polymorphism in the satellite is parallel with the case found by HAGA ('34) in *Paris quadrifolia* var. *obovata*. During the course of the study, the writer has met, though rarely, with the occurrence of two univalents instead of a bivalent in PMCs of *L. dresidensis* (Fig. 12) and this will be assumed to be connected with the heteromorphism in the satellited chromosome pair.



Figs. 9-14, 16 and 17. First meiotic metaphase plates from PMCs. 9. *L. syphilitica* ( $n = 7$ ). 10. *L. inflata* ( $n = 7$ ). 11. *L. dresidensis* ( $n = 7$ ). 12. *L. dresidensis*. Six bivalents and two univalents are shown. 13. *L. sessilifolia* ( $n = 14$ ). 14-15. *L. Erinus* No. 2. 14. Polar view showing 22 chromosomes ( $20_{II} + 2_I$ ). 15. Side view in anaphase I showing two univalents. 16. *L. Erinus* No. 1. ( $n = 21$ ). 17. *L. triquetra* No. 2. ( $n = 7$ ).  $\times 3500 \times 2/3$ .

Concerning the higher polyploids of *Lobelia*, it was not easy to analyse the chromosome types with certainty, but as far as *L. sessilifolia*, a tetraploid species, is concerned, it seems that there are only one pair of satellite-chromosomes and only one pair having two constrictions. This will have significance in considering the origin of such polyploids.

## ii) Some further remarks on *L. sessilifolia*

There being an abundance of good preparations, *L. sessilifolia* was subjected to further special observations, namely, (a) upon the mode of chromosome (bivalent) arrangement at the first meiotic metaphase plate and (b) upon the relationship of chromosomes to nucleoli. Though the results on these points are only of a preliminary nature, they may merit publication as supplying material for further research in these fields.

a) Chromosome arrangement. The mode of arrangement of

the total 14 bivalents of *L. sessilifolia* at the first metaphase was classified into the following three types:

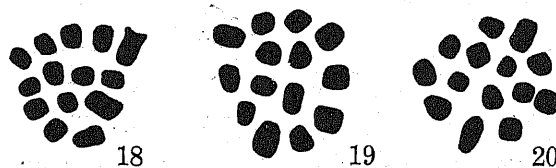
- i) 11 bivalents lying outside the plate, the remaining 3 inside,
- ii) 10 bivalents lying outside the plate, the remaining 4 inside,
- iii) 9 bivalents lying outside the plate, the remaining 5 inside.

These three types are illustrated in Figs. 18, 19 and 20 respectively.

The observed frequencies of these three types in the total 48 PMCs were found to be as follows:

	Frequency	Percentage
i	1	2.08 %
ii	31	64.58
iii	16	33.33

The above data will suggest that the second mode of arrangement is a stable form, a condition to be expected from the floating magnet theory of chromosome arrangement (cf. KUWADA '29).



Figs. 18-20. Illustrating the three types of chromosome arrangement in *L. sessilifolia*.  $\times 3500 \times 2/3$ .

b) The relationship of chromosomes to nucleoli. A very unique behavior of nucleoli was found in the somatic division of *L. sessilifolia*. Features of this matter

may be described in the order of events as follows:

i) At prophase there is only one nucleolus in each cell, with which a chromosome is tightly connected distally (Fig. 23).

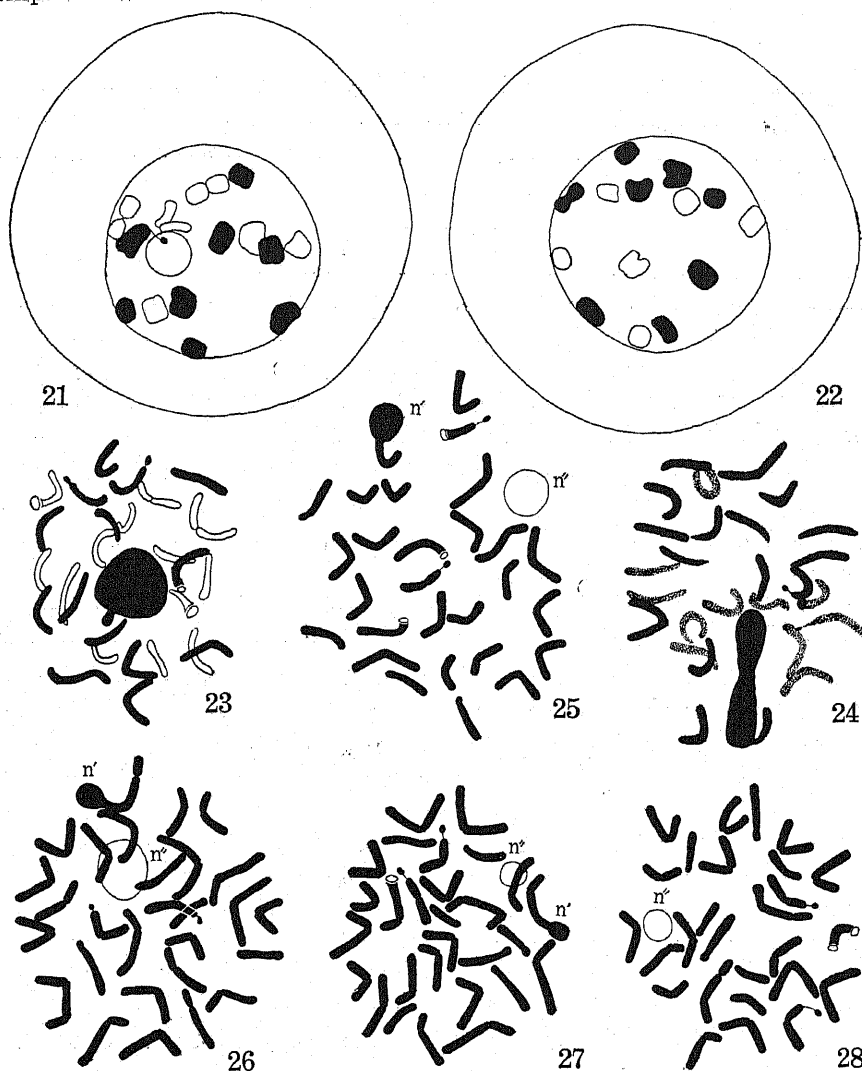
ii) Later on, the nucleolus begins to divide into two parts (Fig. 24).

iii) At stages just prior to metaphase, there are two nucleoli, one of which is connected with a chromosome, while the other is completely free from any chromosome (Figs. 25-27).

iv) The nucleolus attached to a chromosome decreases gradually in volume as the stage proceeds, and at such a stage it can be recognized that the chromosome in question is one of the double-constricted ones.

v) Often the nucleolus which bears no relation to the chromosome remains at metaphase even after the other nucleolus has completely disappeared (Fig. 28). It is a noteworthy fact that in the present material no relationship was observed between the satellite-chromosomes and the nucleolus, but that it is one of the two double-constricted chromosomes which bears the actual relationship to the

nucleolus. The present finding will be interesting in comparison with HEITZ's hypothesis ('31 a, b) on the nucleolus-chromosome relationship.



Figs. 21-22. Two diakinetic nuclei in *L. sessilifolia* showing fourteen bivalents. 21. Representing a bivalent carrying the satellite which has no bearing on the nucleolus. 22. Late diakinesis. The nucleolus has disappeared already.  $\times 3500 \times 2/3$ . Figs. 23-28. Prophase and prometaphase stages in the somatic division of the root tip showing the relation of chromosomes to the nucleolus. 23-24. One large nucleolus is present. 25-27. Two nucleoli  $n'$  and  $n''$  are shown. One of them ( $n'$ ) is in connection with the distal end of one definite chromosome and the other ( $n''$ ) has no relation to any chromosome. 28. Only one of the nucleoli ( $n''$ ) is visible.  $\times 5000 \times 2/3$ .

It was not easy to determine whether parallel behavior of the nucleolus is detectable in the meiotic division. But so far as the

observation goes, it is quite clear that at early diakinesis there is no actual connection of satellite-bivalents with the nucleolus, as illustrated in Fig. 21. At mid-diakinesis usually no nucleolus was visible (Fig. 22).

This work was carried out under the guidance of Professor H. MATSUURA, to whom the writer wishes to express his sincere thanks for kind advices and valuable criticisms throughout the investigation.

### Summary

1) In the present study, seven species of *Lobelia* were dealt with karyologically. They were found to constitute a polyploid series,  $2x-4x-6x$ , with 7 as the basic number; *L. inflata*, *L. dresidensis* and *L. syphilitica* are diploid species, *L. sessilifolia* a tetraploid species, *L. Erinus* and *L. Richardsonii* hexaploid species, and *L. triquetra* contains both diploid and hexaploid individuals.

2) The comparison of idiograms between three diploid species, *L. inflata*, *L. syphilitica* and *L. dresidensis* reveals certain marked differences in the size and structure of their components (cf. Figs. 8 a-c).

3) From a study on the chromosome arrangement at MI of PMCs in *L. sessilifolia*, it was found that the configuration with 4 bivalents lying inside and the other 10 lying outside was prevalent, as the floating magnet theory of chromosome arrangement demands.

4) In the somatic division of *L. sessilifolia*, a unique behavior of the nucleolus was detected. The nucleolus divides at an early stage into two, one of which parts is connected with the distal end of one definite chromosome, the double-constricted one, while the other part is entirely free from any chromosome. No connection was observable between the satellite-chromosomes and the nucleolus in either the somatic and meiotic divisions.

### Literature cited

- Armand, L. 1912. Fécondation et développement de l'embryon chez les Lobéliacées. C. R. Acad. Sci. Paris 155: 1534-6.
- Haga, T. 1934. On the karyotypes and their gametes of *Paris quadrifolia* L. var. *obovata* REGENS et TIL. Bot. Mag. (Tokyo) 48: 241-248.
- Heitz, E. 1931a. Die Ursache der Gesetzmäßigen Zahl, Lage, Form und Größe der pflanzlicher Nukleolen. Planta 12: 775-844.
- 1931b. Nukleolen und Chromosomen in der Gattung *Vicia*. Planta 15: 495-505.
- Kuwada, Y. 1929. Chromosome arrangement 1. Mem. Coll. Sci. Kyoto, S. B. 4: 199-264.
- Vilmorin, R. De. et M. Simonet. 1927. Nombre des chromosomes dans les genres *Lobelia*, *Linum* et chez quelques autres espèces végétales. C. R. Soc. Biol. Paris 96: 166-8.

## Die Gattung *Veronica* in entwicklungsgeschichtlicher Betrachtung

Von

Ernst Lehmann, Tübingen

Wenn ich heute in die Reihen der Gratulanten trete, die dem Meister der Cytologie im fernen Osten zum 70. Geburtstage ihre Glückwünsche überbringen, dann ist es mein Wunsch, ich möchte ihm eine Gabe vorlegen können, auf der sein Auge nicht ganz ohne Interesse verweile. Wird es aber dem Jubilar etwas Freude bereiten, wenn er sieht, wie die entwicklungsgeschichtliche Forschung in der Gattung *Veronica*, die ich nun von Jugend an betreibe, und die meine Schüler bis zum heutigen Tag, teils in Verbindung mit mir, teils durchaus selbstständig weiterpflegen, auf sehr verschiedenen Wegen ihre Krönung und bestmögliche Klärung von der Cytologie her findet, dem Gebiet, auf dem gerade er mit seinen Landsleuten so besonders bahnbrechend gewirkt hat?

Es kann nur eine Skizze unserer Arbeit sein, die ich hier vorlege; nur einige markante Linien seien gezeichnet. Ein abgeschlossenes Ganzes kann noch nicht geboten werden. Die Wege, welche die Entwicklung in der Gattung *Veronica* eingeschlagen hat, sind noch längst nicht alle geklärt. Die Arbeit aber wird weder von mir, noch von denen, die nach mir kommen, so bald abgeschlossen werden können. Drum sei heute diese erste Skizze unserer gemeinsamen Arbeit in der Gattung *Veronica* gezeichnet und Professor K. FUJII zum 70. Geburtstage zugeeignet.

Die Arbeit begann an der Gruppe *Agrestis*. Die Arten dieser Gruppe zogen zunächst dadurch meine Aufmerksamkeit auf sich, daß sie mir Schwierigkeiten bei der Bestimmung machten. Das war schon in meiner Schüler- und Studentenzeit der Fall. Als ich dann die Arten in den Herbarien studierte, stellte sich heraus, daß es anderen ebenso gegangen war, wie mir. Es herrschte damals eine heillose Verwirrung in dieser Gruppe in den Herbarien der ganzen Welt. Bei sorgfältigem, kritischen Studium ergab sich aber alsbald, daß sich die einzelnen Arten scharf von einander unterscheiden und daß die trennenden Merkmale zu einem großen Teil schon durchaus klar heraus gestellt worden waren. Die diagnostische Arbeit war aber noch nicht genügend in die Floren eingedrungen, so daß bei deren Benutzung immer wieder Fehlbestimmungen zustandekommen

mußten. Wenn dies z.T. zweifellos mit der großen Ähnlichkeit der einzelnen Arten und ihrer nicht unerheblichen Modifikabilität zusammenhing, so beruhte es doch auch besonders auf der differenten geographischen Verbreitung der Arten. Ihrer Klarstellung widmete ich mich zunächst. Es ergab sich, daß *V. agrestis* L. vor allem eine Pflanze kühlerer Klimate und größerer Höhenlagen ist; *V. polita* Fr. dagegen dringt in den Niederungen weit bis in südliche Verbreitungsgebiete vor, während *V. opaca* Fr. in Europa eine vorzüglich kontinentale Verbreitung bis tief nach Rußland hinein aufweist (L. 1907b, 1908). Darüber hinaus ergab sich, daß *V. Tournefortii* Gm. erst in geschichtlicher Zeit weite Wanderungen von der kaukasischen Heimat über die alte und neue Welt, begünstigt durch Verschleppung als Ackerunkraut, angestellt hatte (L. 1906), während die vielfach mit ihr verwechselte, ebenfalls im Kaukasus beheimatete *V. filiformis* Sm. im Großen und Ganzen auf das Heimatgebiet beschränkt geblieben ist. In einer Übersichtskarte wurde die Verbreitung der Arten dargestellt (L. 1908).

Ich ging dann dazu über, die Arten zur Erfassung von Variabilität und Modifikabilität in Kultur zu nehmen. Durch Erziehung unter verschiedenen Bedingungen wurde die Modifikationsbreite ermittelt, was zur klaren Erfassung und Abgrenzung der Arten von besonderer Wichtigkeit wurde. Es wurde auch versucht, Bastarde zwischen den einzelnen Arten zu erstellen, was aber mißlang (L. 1914). Dagegen konnten verschiedene Unterarten heraus differenziert werden; von diesen wurden für meine fernere Arbeit besonders wichtig die beiden Unterarten von *V. Tournefortii*, die ich als subsp. *Corrensiana* und subsp. *Aschersoniana* beschrieb (L. 1909a).

Innerhalb der beiden genannten Unterarten fiel nun aber alsbald eine sehr starke Variabilität in der Zahl der Blütenglieder auf. Schon BATESON (1891, 1898) hatte sich mit diesen Variabilitätsverhältnissen, insbesondere mit der Variabilität der Zahl der Blumenblätter beschäftigt. Er fand vor allem fünfblättrige Kronen, teils mit verdoppeltem hinteren, teils mit verdoppeltem vorderen Kronblatt, daneben mancherlei andere Abweichungen vom normal vierzähligen Kronenbau. Diese Varianten traten einmal auf der einzelnen Pflanze in sehr verschiedenen Prozentsätzen auf; z.a. aber ließ sich zeigen, daß in ihrem Variantengehalt verschieden reiche Rassen vorkommen, sogenannte „Zwischenrassen“ im Sinne von DE VRIES. Ich habe die Variabilitätsverhältnisse solcher Zwischenrassen der *V. Tournefortii* sehr eingehend untersucht. Vor allem habe ich dabei meine Aufmerksamkeit der nicht weniger seltenen Variabilität der Kelchblattzahl zugewandt. Hier sind es im besonderen häufig Blüten mit fünftem, median hinteren Kelchblatt, welche zur Beobachtung kommen.

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Auch hier liessen sich Rassen mit verschieden hohem Prozentgehalt solcher pentasepaler Kelche beobachten; es ließ sich zeigen, daß die Varianten in sehr bestimmt periodischer Verteilung über die einzelnen Individuen auftreten und daß die Variabilität im Kelch und Blumenblattkreise korrelativ verknüpft ist. (L. 1909b, 1919a).

Das Auftreten der Pentasepalie mit median hinterem fünften Kelchblatt in Korrelation mit dem Auftreten fünfblättriger Kronen mit verdoppeltem hinteren Kronblatt erschien mir nun aber deshalb von besonderem Interesse, da hier ein Merkmal von stammesgeschichtlicher Bedeutung in seinen Variationsverhältnissen erfaßt war (L. 1919a). Die vierzähligen *Veronicablüten* sind ja zweifellos abgeleitet von dem fünfzähligen Grundbau der Scrophulariaceenblüte. Das hintere Kronblatt ist häufig noch zweinervig im Gegensatz zur Einnervigkeit der übrigen Kronblätter der *Veronicablüte* und auch im Gegensatz zu dem Verhalten vieler anderer *Veronica*arten, bei denen die Ableitung soweit gegangen ist, daß auch das hintere Kronblatt nunmehr einen Nerv aufweist (JUEL 1891, SAUNDERS 1934). Auch die Pentasepalie tritt ja in manchen Verwandtschaftsgruppen der Gattung noch sehr regelmäßig auf, während sie in anderen Verwandtschaftsgruppen ganz oder fast ganz fehlt. Dabei zeigte sich die Pentasepalie in den einzelnen Fällen nicht selten ontogenetisch verschieden begründet (FISCHER 1920). Gelang es also über den Erbgang dieser Pentasepalie Aufklärung zu erlangen, so war damit auch etwas für die Phylogenie der Gattung, wie darüber hinaus der ganzen Scrophulariaceenreihe gesagt (L. 1919a).

Mit dieser Zielsetzung ging ich an die Erstellung von Kreuzungen zwischen Rassen mit verschiedenem Pentasepaliegehalt heran. Für die ausschlaggebenden Verbindungen kamen die folgenden drei Biotypen zur Verwendung: 1.) Ein Biotyp von *V. Corrensiana* mit nahezu 100% vierblättrigen Kelchen; 2.) Ein Biotyp von *V. Aschersoniana* mit etwa 90% vierblättrigen Kelchen; 3.) Ein Biotyp, den ich aus Kreuzungen verschiedener *Aschersoniana*-Formen erzielt hatte mit nahezu 100% fünfblättrigen Kelchen, dem ich den Namen *V. tubingensis* beilegte. Bei Kreuzung dieser Biotypen untereinander ergab sich zunächst die folgende interessante Tatsache: Die beiden tetrasepalen oder nahezu tetrasepalen Biotypen verhielten sich der pentasepalen *V. tubingensis* gegenüber insoferne verschieden, als sich die Tetrasepalie von *V. Corrensiana* der Pentasepalie der *V. tubingensis* gegenüber rezessiv, die Tetrasepalie von *Aschersoniana* aber dieser gegenüber dominant zeigte. Aus dieser Tatsache ließ sich zunächst, unter Berücksichtigung der reichlich vorhandenen individuellen Variabilität der Zahl der Kelchblätter das beständige Umschlagen derselben, die Schwierigkeit des Auslesens nach der Zahl

der Sepalen in einer Richtung verstehen; man hat es ja in einem Falle mit dominanter, im anderen mit rezessiver Pentasepalie bzw. Tetrasepalie zu tun, ohne dem Merkmal äußerlich das Erbverhalten ansehen zu können.

Handelt es sich nun aber bei der rassenmäßigen Verteilung des Prozentgehaltes der Pentasepalie um einfach erfaßbare mendelnde Gene? Die Versuche zeigten, daß dies nicht der Fall ist. Die Verhältnisse waren zunächst so undurchsichtig, daß sie Anlaß zu den verschiedensten Erklärungsversuchen boten (L. 1914). Erst durch sehr eingehende Studien (L. 1921b) konnten einigermaßen befriedigende mendelistische Erklärungsversuche mit Hilfe mehrerer gleichsinnig verschieden stark wirkender Faktoren, die ich in vorläufiger Form in die Chromosomen zu verlegen versuchte, erbracht werden. Wenn auch die in meinen Kreuzungen und Rückkreuzungen erhaltenen Zahlenverhältnisse so noch keine Klärung in allen Einzelheiten fanden, so war doch der Weg gewiesen, das in phylogenetischem Sinne so interessante Merkmal der Pentasepalie erbbiologisch zu erfassen. Wie dann später mein Schüler BEATUS auf diesen Grundlagen in Verbindung mit eigenen cytologischen Arbeiten erfolgreich weiter vorgedrungen ist, wird alsbald darzustellen sein.

Vorerst aber war überhaupt die cytologische Grundlage in der Gattung zu legen. Als ich meiner Schülerin, Fräulein HUBER, 1925 nahelegte, die Chromosomenzahlen in der Gattung *Veronica* und insbesondere auch in der Gruppe *Agrestis* klarzustellen, lag nach dieser Richtung in unserer Gattung überhaupt noch nichts vor. Es erschien dann aber zunächst 1926 eine Arbeit von HEITZ, in welcher von *V. opaca* die Haploidzahl auf 12–14 Einheiten angegeben wurde. Einwandfrei konnte dann HUBER (1927) für *V. polita*  $n = 7$  feststellen und für *V. Tournefortii*  $n = 14$  sehr wahrscheinlich machen, so daß 7 als Grundzahl für die Gruppe *Agrestis* anzunehmen war und für *V. Tournefortii* Tetraploidie auf dieser Basis nahegelegt war. Diese Feststellungen wurden dann richtungsgebend für die weiteren Untersuchungen von BEATUS (1934, 1936a). Er stellte die folgenden Zahlen einwandfrei fest: *V. polita*  $n = 7$ , die nahverwandten Arten *agrestis* und *opaca*  $n = 14$ ; *V. Tournefortii*  $n = 14$ , die dieser naheverwandte *V. filiformis*  $n = 7$ . Auch SIMONET (1934) gibt diese Zahlen für die letztgenannten beiden Arten an.

Unterdressen war ja nun aber die Cytologie, vor allem auch die der polyploiden Formen sehr viel weiter fortgeschritten. Unter glücklicher Auswertung so erlangter Erkenntnisse und auf Grund eigener, vertiefter cytologischer Untersuchungen an den Arten der Gruppe konnte BEATUS (1934) die Zwischenrassenfrage bei *V. Tournefortii* noch viel befriedigender klären als mir dies ohne cytolo-

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gische Studien gelungen war. Auf Grund häufig auftretender Quadrivalenter während der Reifeteilung kommt BEATUS zur Annahme von Autopolyploidie für *V. Tournefortii*; werden dann weiter die Pentasepaliefaktoren als multiple Allele aufgefaßt, so lassen sich die in meinem früheren, wie in den später von BEATUS angestellten Kreuzungen erhaltenen Zahlenwerte sehr wohl verständlich machen. Mancherlei Einzelfragen würden dann noch auf Grund entwicklungsgeschichtlicher Untersuchungen der *Veronica*-Blüte, wie sie von meinem Schüler FISCHER (1920) in Angriff genommen worden waren, beantwortet werden können.

So wäre dieses, auf einer großen phylogenetischen Reduktionsreihe liegende Merkmal der genotypischen Klärung nahegebracht.

BEATUS (1936 a, c) aber führte die chromosomalen Studien in der Gruppe *Agrestis* dann noch weiter durch und wertete sie in entwicklungsgeschichtlichem Sinne aus. Er findet verschieden häufige Multivalenz bei den drei polyploiden Arten der *Agrestis*-Gruppe. Am geringsten ist die Zahl der multivalenten Chromosomen bei *V. agrestis* selbst. Es wird daraus geschlossen, daß der Verwandtschaftsgrad zwischen den entsprechenden Chromosomen der vier Genome nicht sehr hoch ist und daß die Entstehung der Art verhältnismäßig weit zurückliegt. Bei *V. Tournefortii* sind multivalente Chromosomen und „secondary association“ häufiger zu finden. Demnach zeigen die Genome noch einen hohen Verwandtschaftsgrad. Die Entstehung der *V. Tournefortii* wäre somit jüngeren Datums. Entsprechendes gilt für *V. opaca*. Auf Grund dieser seiner caryologischen Feststellungen macht sich nun BEATUS in Verbindung mit meinen früheren pflanzengeographischen Studien ein Bild von dem Entwicklungsgeschehen in der Gruppe *Agrestis*. Im Gebiete der Heimatgruppe, dem Kaukasus und Persien, sind die beiden diploiden Arten *polita* und *filiformis* vertreten, außerdem die tetraploide *V. Tournefortii*. Für *V. Tournefortii* wird nun Entstehung aus *V. filiformis* durch Chromosomenverdoppelung und damit Autotetraploidie angenommen, wie wir schon weiter oben erwähnten. *V. Tournefortii* hat die Heimat erst in jüngerer Zeit verlassen, *V. polita* aber ist von dort schon lange auf die Wanderschaft gegangen und zu ihrer weltweiten Verbreitung gekommen. Wie aber ist die Entstehung von *V. agrestis* und *V. opaca* zu denken? Beide kommen in der Heimat der übrigen drei Arten nicht vor. BEATUS nimmt für sie Nordeuropa als wahrscheinliches Entstehungsgebiet und *V. polita* als Ursprungsart an. Für diese verschiedene Entstehungsgeschichte spricht auch besonders die nahe Verwandtschaft der Arten der beiden jeweiligen Gruppen. Zudem würde auch noch das Vorkommen von *V. siaretensis* LEHMANN, welche Merkmale von *V. Tournefortii* und *V. polita* in sich vereinigt,

in Persien für die Annahme von BEATUS sprechen. Die Entstehung der polyploiden *V. agrestis* und *opaca* fern vom Gebiet des ursprünglichen Vorkommens harmoniert aber auch mit den interessanten Feststellungen von HAGERUP (1933) und TISCHLER (1935), nach denen Pflanzen, die von ihrer ursprünglichen Heimat in neue Gebiete vordringen, dort infolge der abweichenden klimatischen Bedingungen Störungen im Ablauf der Reifeteilung aufweisen, die zu polyploiden Formen führen. Mancherlei Einzelfolgerungen können hier nicht erörtert werden.

So aber zeigt sich, wie systematische, pflanzengeographische, entwicklungsgeschichtliche, genetische und schließlich cytologische Forschungen gemeinsam immer tiefer in die Kenntnis der Entwicklungsgeschichte dieser Gruppe eingeführt haben und uns somit den Zielen näher brachten, welche mir bei Inangriffnahme der Arbeit vorschwebten. Schon aber wird von BEATUS der Weg weitergewiesen, wie zum Beweis der gemachten Annahmen die Verdoppelung der Chromosomen etwa bei *V. filiformis* oder *V. polita* experimentell in Angriff genommen werden könnte.

Als ich 1907 mit meiner ersten *Veronica*-Arbeit „Über Wanderung und Verbreitung von *V. Tournefortii*“ in Dahlem Geheimrat ENGLER begegnete, schlug dieser dem damals neuauftauchenden jungen Botaniker vor, die *Veronica*-Studien zu erweitern und die ganze Gattung für sein „Pflanzenreich“ zu bearbeiten. Diese Anregung bestärkte mich, die schon in Angriff genommene systematische und pflanzengeographische Durchforschung weiterer Gruppen mit besonderer Energie fortzusetzen. So dehnte ich meine Arbeit zunächst auf die benachbarten Gruppen der Sektion Alsinebe, der auch die Gruppe *Agrestis* angehört, aus. Es erwies sich hierbei zunächst wesentlich, die Arten dieser Sektion in einzelne, natürliche Gruppen schärfer zusammenzufassen. Ich kam zu einer Gruppeneinteilung, über die ich in meiner Arbeit über die *Veronica*-Sektion Alsinebe (1910) berichtete. Daneben aber machte ich die Feststellung, daß in den Gruppen der ganzen Sektion dieselben Merkmale in den verschiedensten Kombinationen einmal hervor, das andere Mal zurücktraten. „Man gewinnt danach den Eindruck, daß diese Merkmale auf Merkmalseinheiten oder Anlagen basieren, die mehr oder weniger unabhängig voneinander sich verändern können, wie es in neuester Zeit durch die MENDELSchen Bastardierungsversuche in anderem Zusammenhange nachgewiesen wurde. Die Entwicklung der Arten kann hier also nicht einfach als Ganzes betrachtet werden, sondern die Einzelmerkmale und deren Verhalten müssen ins Auge gefaßt werden, wenn man eine natürliche Anordnung der Arten wünscht.

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Gerade Entwicklungsreihen lassen sich aus diesem Grunde hier ganz und gar nicht konstruieren. Wir kommen vielmehr zu der Anschauung, daß beim Zustandekommen der Artenmannigfaltigkeit in der Sektion Alsinebe eine relativ geringe Menge von erblichen Einheiten mitgewirkt hat, deren wechselweises Hervortreten und Zurücktreten, jedenfalls verbunden mit gegenseitigem Aufeinanderwirken dann zu dem Bild geführt hat, welches uns heute die Sektion zeigt.“

Immer aber zogen mich Einzelprobleme und -Beobachtungen in ihren Bann und rückten das Ziel der systematischen Gesamtbearbeitung der Gattung in nebelhafte Ferne. Aus der Sektion Alsinebe kam mir *V. syriaca* in die Hand. Eine unerhörte Variabilität in der Zahl der Blütenglieder trat mir entgegen und forderte zu statistischen, wie Korrelationsuntersuchungen auf (L. 1917). Bei den zur erbbiologischen Klärung dieser Variationerscheinungen angestellten Kreuzungen blieben nun aber immer einzelne Kombinationen aus und die notwendigen Selbstungen versagten. So konnte ich nicht lange im Zweifel darüber bleiben, daß es sich in *V. syriaca* um eine selbststerile Art handele. Ich war nicht umsonst Assistent bei meinem Lehrer, Professor JOST gewesen, hatte mit Spannung in Leipzig an einem Colloquium teilgenommen, bei welchem PFEFFER und CORRENS zur Frage der Selbststerilität Stellung genommen hatten! CORRENS hatte ja dann das Selbststerilitätsproblem bei *Cardamine pratensis* auf genetischer Basis in Angriff genommen und weitgehend gefördert, ohne es allerdings zum endgültigen Abschluß bringen zu können. Da nahm ich (1919a, 1921a) die Frage der Selbststerilität bei *V. syriaca* auf und konnte sie in Gemeinschaft mit meinem Schüler FILZER (1926) zu befriedigender Lösung bringen. Multiple Allelie war des Rätsels Lösung geworden. Ganz unabhängig hatten wir an *V. syriaca* und East bei *Nicotiana* um die gleiche Zeit dasselbe Ergebnis erzielt.—Selbststerile *Veronicae* aber fanden sich dann auch noch in anderen Gruppen; so stellte ich solche in der Gruppe Pentasepala fest und CORRENS studierte die Selbststerilität von *V. gentianoides*.

Durch herbarkritische und pflanzengeographische Untersuchungen gelang es dann weiterhin, auch tiefer in die Kenntnis der Gruppen *Biloba* und *Megasperma* einzudringen.

Wie wir noch sehen werden, waren aber unterdessen für andere Gruppen der Gattung in meinem Institut angestellte chromosomale Untersuchungen besonders aufschlußreich geworden. So erschien es lohnend, solche Untersuchungen auch in der Sektion Alsinebe über die schon studierte Gruppe *Agrestis* hinaus auszudehnen. Diese Aufgabe übernahm Fräulein HOFELICH (1935). Auffallend waren die sehr verschiedenen Chromosomenzahlen, die hier festgestellt werden

konnten. Einmal kommt hier nicht nur die Grundzahl 7, wie sie für die Agrestis-Gruppe aufgefunden wurde, vor; es traten auch die Grundzahlen 8 und 9 auf, auf denen sich wie auf der Grundzahl 7 polyploide Reihen aufbauten, und es fanden sich daneben auch aneuploide Zahlen. Es folge eine Übersicht der in der Sektion gefundenen

Chromosomenzahlen	n	2n
Verwandschaftsgruppe Megasperma:		
<i>V. sibthorpioides</i> DEB. et DEG.	15	
<i>V. hederifolia</i> L.	28	
<i>V. panormitana</i> TIN.	9	
<i>V. cymbalaria</i> BOD.	18	
Verwandschaftsgruppe Diplophyllum:		
<i>V. cardiocarpa</i> WALP.	7 bzw. 8	15
<i>V. biloba</i> L.	14	
<i>V. campylopoda</i> BOISS.	21	42
<i>V. arguteserrata</i> REG. et SCHM.	21	
Verwandschaftsgruppe Mikrosperma		
<i>V. arvensis</i> L.	8	
<i>V. verna</i> L.	8	
<i>V. Dillenii</i> CRTZ.	8	
<i>V. peregrina</i> L.	26	
Verwandschaftsgruppe Pellidosperma:		
<i>V. triphyllus</i> L.	7	
<i>V. praecox</i> ALL.	9	
Verwandschaftsgruppe Acinifolia:		
<i>V. acinifolia</i> L.	7	
<i>V. syriaca</i> ROEM. et SCHULT.	7	14
Verwandschaftsgruppe Serpyllifolia:		
<i>V. serpyllifolia</i> L.	7	
<i>V. repens</i> DC.	7	

Vergleichen wir die innerhalb der einzelnen Gruppen festgestellten Chromosomenzahlen mit der Einteilung, zu der ich auf Grund der morphologischen Merkmale gelangt war, (L. 1910), so zeigt sich eine bemerkenswerte Übereinstimmung mit der damals durchgeführten Gruppenbildung. Wie wir noch sehen werden, ist die Grundzahl in der ganzen Gattung *Veronica*  $n = 8$ , eine Zahl, welche HEITZ als Grundzahl der Scrophulariaceenreihe feststellte. Die Grundzahlen 7 und 9, die in der Sektion Alsinebe neben der Grundzahl 8 zur Beobachtung kommen, sind wohl ohne Zweifel von der Grundzahl 8 der Gesamtgattung abzuleiten. Von Interesse ist in diesem Zusammenhange, daß *V. cardiocarpa* teils die Haploidzahl 7, teils 8 aufweisen hat, wobei von den 7 Chromosomen das eine größer als die übrigen sein und gelegentlich eine Einkerbung aufweisen kann. Das



legt die Annahme nahe, daß die abgeleitete 7-Zahl aus Verschmelzung von zwei der ursprünglichen acht Chromosomen zustandegekommen sein mag. Umgekehrt wäre die Zahl 9 wohl auf Chromosomenzerfall zurückzuführen. Auf die mancherlei Schlußfolgerungen im einzelnen, etwa die, welche das chromosomale Verhalten in den Gruppen Megasperma und Biloba in Verbindung mit der geographischen Verbreitung nahelegt, u. a. sei hier nicht eingegangen, sondern auf die diesbezüglichen Darlegungen von HOFELICH (1935) und BEATUS (1936a) verwiesen. Von besonderem Interesse dürften in Zukunft Kreuzungsversuche innerhalb der Gruppe Biloba werden. Kreuzungen innerhalb der Gruppe Megasperma dagegen, welche wir hier anstellten, haben bislang noch keine Erfolge gezeitigt.

Wir wenden uns nunmehr zu einer kurzen Betrachtung unserer Untersuchungen innerhalb der Sektion *Veronicastrum*, die aus verschiedenen Gründen als besonders ursprünglich aufzufassen sein dürfte. Hier tritt sehr häufig die Fünffzahl in den Blütenwirteln auf; Pentasepalie ist vielfach vertreten; Fünfnervigkeit der Blumenkrone desgleichen. Ja auch Arten mit regelmäßig fünf Blumenblättern konnte FRIES (1925) aus dem Kilimandscharo-Gebiet bringen. Als regelmäßige Grundzahl der Chromosomen ließ sich ferner die ursprünglich Scrophulariaceenzahl 8 nachweisen. HUBER gab für *V. Gouani* 16–18 Chromosomen an, für *V. gentianoides* 24. Diese Zahl wurde von SIMONET (1934) bestätigt, der daneben interessanter Weise noch eine *V. gentianoides variegata* mit 32–34 Chromosomen fand. Auch *V. fruticulosa* und *fruticans* besitzen  $n = 8$  Chromosomen (SIMONET 1934, BEATUS 1936b). *V. alpina* mit  $n = 7$  Chromosomen SIMONET (1934) wäre wieder abgeleitet.

Von der geographischen Verbreitung der Arten der Sektion *Veronicastrum* brachte HUBER (1929) interessante kartographische Darstellungen, welche wiederum das relativ hohe Alter der Gruppe belegten. Die vorzüglich arktischen und alpinen Formen haben ein sehr weites aber auch vielfach unterbrochenes Gesamtareal.

Genetische Untersuchungen durch Kreuzung der in ihren Verbreitungsverhältnissen so sehr verschiedenen beiden Arten *V. fruticulosa* und *V. fruticans* (HUBER 1929) stellte BEATUS (1936b) an. Während *V. fruticulosa* bekanntlich eine beschränkte alpine Verbreitung hat, ist *V. fruticans* einmal in den Alpen viel weiter verbreitet als jene, kommt darüber hinaus aber auch in Skandinavien vor. BEATUS konnte nun zeigen, daß beide Arten sich leicht untereinander kreuzen lassen, und daß die Bastarde normal fertil sind. Er erwies weiter, daß die Artdifferenzen z.T. auf mendelnden Grundunterschieden beruhen und es leicht ist, Neukombinationen zu erzielen, bei



denen bestimmte Artmerkmale der beiden Spezies ausgetauscht werden können; auch konnten in der Literatur beschriebene Arten und Varietäten dieser Verwandtschaftsgruppe durch Kreuzung von *V. fruticans* und *V. fruticulosa* erstellt werden.

Die Sektion *Chamaedrys* wurde herbarkritisch und pflanzengeographisch schon 1914, teilweise ziemlich weitgehend von WULFF, sehr eingehend aber neuerdings von meinem Schüler RIEK (1935) bearbeitet. Dieser suchte die sehr zahlreichen Arten der Sektion in kleinere natürliche Gruppen zusammenzufassen, die einzelnen Arten in ihren Charakteren und in ihrer geographischen Verbreitung klarzustellen.

Eine stammesgeschichtlich besonders interessante Gruppe dieser Sektion, die Gruppe Pentasepala, wurde in den unerhört formenreichen Arten *Teucrium*, *prostrata* und *austriaca* schon 1910 von WATZL herbarkritisch sorgfältig studiert, sodaß RIEK auf ihre erneute Behandlung verzichten konnte. In dieser Gruppe ist, wie schon der Name sagt, die Pentasepalie besonders stark, wenn auch keineswegs ausnahmslos vertreten (L. 1914). Auch sonst besitzt sie mancherlei ursprüngliche Züge. Experimentell-cytologische Untersuchungen innerhalb dieser Gruppe begann mein Schüler SCHEERER (1937).

Die Kreuzungsuntersuchungen erwiesen sich hier allerdings wegen der zumeist vollkommenen oder doch sehr weitgehenden Selbststerilität und dem perennierenden Charakter der Arten recht schwierig und zeitraubend. So beschränkt sich die experimentelle Bearbeitung zunächst nur auf die Feststellung der Kreuzungsmöglichkeiten zwischen den einzelnen Arten und zahlreichen Unterarten. Auch die Beschreibung der Bastarde konnte noch nicht voll durchgeführt werden.

Gerade die Frage der Kreuzungsmöglichkeit aber erwies sich von besonderem Interesse und wurde eingehend verfolgt. Es ergab sich eine weitgehende Beschränktheit der Kreuzungsmöglichkeit; gut kreuzbar sind nur: *V. austriaca* ssp. *dentata* und ssp. *Jacquini*; *V. austriaca* ssp. *orbiculata* und *V. prostrata* Schwaben; *V. Teucrium* ssp. *pseudochamaedrys* und *V. austriaca* ssp. *dentata* und ssp. *Jacquini*, wobei bemerkenswert ist, daß bei Kreuzung auch zwischen verschiedenen Varietäten derselben Art Störungen in der Samenbildung auftreten können. Bei der morphologischen Einheitlichkeit der Gruppe und den vielfach vermuteten Bastarden war dieser Befund zunächst überraschend. Jedenfalls erweist er, daß die Frage, inwieweit Kreuzungen an den in der Natur aufgefundenen Zwischenformen beteiligt sind, stets nachzuprüfen ist.

*Lehm*  
CA  
7. 1937

Weitgehende Klarheit über die Grundlage der verschiedenen Kreuzungsmöglichkeiten erbrachte die cytologische Untersuchung; es ergab sich nämlich die folgende polyploide Reihe aus den Untersuchungen SCHEERERS:

	n	2n
<i>V. prostrata</i> Ungarn	8	16
<i>V.</i> „ Schwaben	16	32
<i>V. austriaca</i> ssp. <i>dentata</i>	24	48
„ „ ssp. <i>Jacquini</i>	—	48
„ „ ssp. <i>orbiculata</i>	—	32
„ „ <i>Teucrium</i> ssp. <i>pseudochamaedrys</i>	—	64

Werden die cytologischen Ergebnisse mit den experimentell festgestellten Kreuzungsmöglichkeiten verglichen, so zeigt sich, daß nur diejenigen Formen normal kreuzbar sind, welche gleiche Chromosomenzahlen aufweisen. Bei den Kreuzungen zwischen verschiedenen chromosomigen Eltern können ausnahmsweise vereinzelte Samen angesetzt werden, bislang zudem auffallenderweise nur bei denjenigen mit höherchromosomigem Elter als Mutter. Die Regel geht soweit, daß aus der Kreuzbarkeit bzw. Nichtkreuzbarkeit weitgehend auf die Chromosomenzahl geschlossen werden kann.

Auf die Feststellungen der Chromosomenzahlen in dieser Gruppe durch HUBER (1927) und SIMONET (1934), die von den von SCHEERER gewonnenen Ergebnissen nicht abweichen, sei nur verwiesen.

In den übrigen zur Sektion *Chamaedrys* gehörigen Gruppen fehlen genetische Untersuchungen noch vollständig, wiewohl sie z.B. innerhalb der so überaus vielförmigen Gruppe *Orientalis* (vgl. RIEK 1935) sehr viel Wesentliches versprechen. In der Gruppe *Multiflora* stellte GOTTSCHICK (unveröfftl.) die Zahlen 8 und 16 fest. Nach SIMONET (1934) finden sich bei *V. officinalis*  $2n = 34-36$ , *Chamaedrys*  $n = 16$ , *montana*  $n = 9$ , also die Grundzahl 8 oder die von ihr abgeleitete Zahl 9.

Von besonderem Interesse erschiene auch die Klarstellung der chromosomalen Verhältnisse von *V. javanica* BL., die ich als Ubiquist tropischer und subtropischer Gebirge feststellen konnte, die aber in ihrer systematischen Stellung noch sehr umstritten ist (L. 1912).

Die Gruppe *Beccabunga* wurde von meinem Schüler SCHLENKER (1936 a, b) im hiesigen Institut herbarkritisch, pflanzengeographisch, genetisch wie cytologisch aufs eingehendste untersucht. Auch in dieser Gruppe besteht große Formenmannigfaltigkeit, die zu verwirrenden systematischen Verhältnissen, zur Beschreibung vielfältiger Kleinformen den Anlaß bot. Bei diesen Uferpflanzen bilden zunächst modifikatorische Einflüsse eine bedeutsame variationsbildende Ur-

sache. Z.a. aber treten auch sehr zahlreiche, erblich fixierte Kleinarten auf. Durch die weitgehende Selbstfertilität und die ausgesprochene Kreuzungssterilität bei morphologisch nahestehenden Formen ist es möglich, daß die vielfältigen Formen nebeneinander auftreten und sich als solche erhalten können. Kreuzungen zwischen Kleinsorten derselben Art (*V. Anagallis*), welche, wenn auch nicht voll fertile Bastarde ergeben, zeigen in der  $F_2$  eine verwirrende Mannigfaltigkeit der Aufspaltung, in welche nur mit Mühe mendelistische Grundlagen eingeführt werden können.

Die Haploidgrundzahl der Gruppe ist 9, wobei *Beccabunga*, *anagalloides* und *oxycarpa* die Zahl 9, *Anagallis*, *aquatica* und *americana* die Zahl 18 aufweisen. (vgl. dazu HUBER 1927, SIMONET 1934). Welcherlei Klärung die cytologische Forschung hier noch bringen kann, bleibt abzuwarten. Zweifellos aber spielen Faktormutationen bei der Variationsbildung in dieser Gruppe eine besondere Rolle.

Eine fast unübersehbare Formenmannigfaltigkeit bieten nun aber auch die vielfach durch Übergänge verbundenen, biotypenreichen Arten der Sektion *Pseudolysimachia*. An ein Durchfinden durch diese zahllosen Formen oder gar ein Abgrenzen und Erfassen derselben war bislang überhaupt noch nicht zu denken, trotz der unzähligen Untersuchungen, die dieser Sektion gewidmet waren. Ich ging zunächst daran, durch HUBER (1929) die geographische Verbreitung der Hauptarten erfassen zu lassen, da ich ja bei anderen Gruppen hiermit mancherlei Erfolge gehabt hatte. HUBER fand einmal vielfach sich überdeckende Großareale der Hauptarten (*V. longifolia*, *spicata*, *spuria*, *incana*), neben mancherlei Arten mit beschränkten Verbreitungsgebieten (*V. Komarovii*, *pinnata*, *laeta*, *grandis*, *Bachofeni*, *sajanensis* u.a.). Eine Klarstellung der vielen Kleinarten war aber auf diesem Wege nicht zu erreichen. So übernahm Herr HÄRLE die Aufgabe, die Gruppe herbarkritisch, pflanzengeographisch und weiterhin auch genetisch aufzuhellen. „Wie in wenigen Formenkreisen“, sagt HÄRLE, „wechseln hier die Auffassungen darüber, wie weit der Umfang der einzelnen Arten zu ziehen ist—kleine Arten treten immer erneut hervor und werden wieder—wegen mangelnder scharfer Begrenzungsmöglichkeit—eingezogen.—Es hiesse das Spiel von neuem wiederholen, wollte man versuchen, allein durch herbarkritische Untersuchungen zum Ziele zu kommen. Scharfsinnige Beobachter haben hier schon ihr Möglichstes versucht und sind dann doch von anderen nicht weniger scharfsinnigen Autoren widerlegt worden.—Um in dieser Gruppe Klarheit zu schaffen, mußte man versuchen, alle Wege zu gehen, welche zu diesem Ziele zu führen versprochen. Vergleichende geographische Forschung, Kulturversuche, Kreuzungs-

studien und physiologische Untersuchungen mußten hier einsetzen, nachdem, das Herbarmaterial auf möglichst umfangreicher Basis kritisch gesichtet war. Auf diese Weise wurde versucht, dem Verständnis der Formenmannigfaltigkeit in der Sektion *Pseudolysimachia* näher zu kommen“.

Und so hat HÄRLE die Gruppe auf all diesen verschiedenen Gebieten mustergültig untersucht. Besonders wesentlich war es, daß diesen Untersuchungen zu einem großen Teil nicht Gartenmaterialien zu Grunde gelegt werden mußten, sondern solche von den natürlichen Standorten beschafft werden konnten. Diese wurden von HÄRLE entweder selbst, auch von ferne, herbeigeschafft, oder von Kollegen freundlichst vermittelt.

Als Grundlage der Untersuchungen diente eine sehr eingehende Behandlung und Umschreibung der Grundarten mit ihren Formenkreisen. Bei der experimentellen Bearbeitung ergab sich aber zunächst, daß die meisten der untersuchten Formen dieser Sektion bei Selbstbestäubung mehr oder weniger weitgehende Inzuchtreduktion aufwiesen, die sich in schlechtem Samenansatz und langsamem Wachstum der aus diesen erzeugten Pflanzen ausdrückt. Die Bastarde dagegen erwiesen sich, sofern sie herstellbar waren, sowohl in ihrem Wachstum, als in ihrer Fertilität, besonders kräftig, auch den reinen Arten gegenüber. Höchst bedeutsam war aber, daß, wie in dieser Gruppe zuerst festgestellt wurde, die Kreuzungsmöglichkeit zwischen den einzelnen Arten eine sehr verschiedene war, und daß sich sämtliche Arten in zwei Gruppen einteilen ließen, deren Angehörige unter sich kreuzbar, aber mit denen der anderen Gruppe nicht kreuzbar waren.

Die beiden Gruppen sind die folgenden:

- |                         |   |
|-------------------------|---|
| 1. <i>V. longifolia</i> | 2. <i>V. spuria</i>                                       |
| <i>V. spicata</i>       | <i>V. foliosa</i>   |
| <i>V. crassifolia</i>   | <i>V. Bachofeni</i>                                       |
| <i>V. incana</i>        | <i>V. maritima</i>  |
|                         | <i>V. orchidea</i>  |
|                         | <i>V. spicata</i> S <sub>1</sub> (bzw. <i>V. euxina</i> ) |
|                         | <i>V. Barrelieri</i> .                                    |

Die F<sub>2</sub> erwies sich bei den meisten Bastarden auffällig konstant und von der F<sub>1</sub> nicht unterschieden. In einzelnen Fällen traten reziproke Verschiedenheiten auf, auf die wir noch zurückkommen. Daß unter den oben geschilderten Verhältnissen die Bastardierung an der Hervorbringung der Kleinformen einen großen Anteil hat, ist nicht zu bezweifeln und durch HÄRLE's Untersuchungen in vielen Einzelfällen belegt.

Aber auch hier wieder konnten erst die chromosomalen Studien befriedigende Klärung der Ergebnisse in verschiedenen Richtungen erbringen. In unermüdlicher Sorgfalt hat Fräulein GRAZE (1932) die Chromosomenzahlen von überaus zahlreichen Arten der Sektion studiert. Es konnten Arten mit  $n = 17$  und  $n = 34$  festgestellt werden. Und nun wurde der Grund der verschiedenen Kreuzungsmöglichkeit klar: Leicht kreuzbar waren die Arten bzw. Formen mit gleichen Chromosomenzahlen, nicht oder schwer kreuzbar die mit verschiedenen Chromosomenzahlen.

Daneben aber ergab sich eine weitere, besonders interessante Tatsache; bei der Untersuchung einiger Varietäten von *V. longifolia* wurden in den Pollenmutterzellen neben 32 gleichgroßen Bivalenten zwei Gemini von besonderer Größe beobachtet. Es war danach anzunehmen, daß 34 Chromosomen durch Verschmelzung von zwei Einheiten zustandegekommen waren. Entsprechendes wurde in weiteren Fällen, wie auch bei 17 chromosomigen Formen (*V. maritima* u.a.) festgestellt, wo dann jeweils das 17. Chromosom sich größer zeigte und aus zwei Einheiten hervorgegangen sein dürfte. Diese Annahme fand eine Stütze dadurch, daß GRAZE (1935) unter einer Kultur von *V. longifolia* auch eine Pflanze mit 36 gleich großen Chromosomen feststellen konnte. Die Grundzahl 17 möchten wir uns daher in dieser Sektion auf Grund ihrer Stellung in der Gesamtgattung und im Hinblick auf die besonders bei Formen der *V. maritima* und *V. longifolia* beobachteten großen Chromosomen durch Chromosomenverschmelzung aus 18-chromosomigen Typen entstanden denken. Die eigentliche Grundzahl in dieser Sektion wäre somit wohl 9, die ihrerseits aus dem Zerfall eines Chromosoms der Grundzahl 8 der Gattung hervorgegangen wäre.

Von den übrigen Ergebnissen ist weiterhin von Interesse, daß aus der Kreuzung verschiedenchromosomiger Formen teils fertile, teils sterile Bastardpflanzen erhalten wurden. Dabei erwiesen sich die fertilen Pflanzen entgegen der Erwartung als tetraploid, die sterilen dagegen als triploid. Bezüglich der Reduktionsteilung wurden in den Pollenmutterzellen der triploiden Bastarde, die auch sonst bei Triploiden gefundenen Besonderheiten beobachtet (Trivalente, Univalente, Restitutionskerne usw.), während sich die Reduktionsteilung der fertilen tetraploiden Bastarde in der gleichen Weise wie bei den tetraploiden Elternformen darstellte.

Von züchterischem Interesse zeigte sich dann noch das Folgende: Hinsichtlich des Kreuzungserfolges war es nicht gleichgültig, ob die höher- oder niederchromosomige Form als Mutterpflanze verwendet wurde. Wie auch bei vielen anderen Pflanzengattungen fielen die Kreuzungsergebnisse mit niederchromosomigen Mutterpflanzen im all-

gemeinen günstiger aus. Die fertilen, also tetraploiden Bastarde wurden meist aus der Verbindung ( $17 \times 34$ ) erhalten, während aus der Kreuzung ( $34 \times 17$ ) in der Regel sterile, also triploide Pflanzen hervorgingen.

Die Gesamtergebnisse aus den verschiedenen Kreuzungen lassen dies deutlich erkennen: von 36 erhaltenen Bastarden aus der Verbindung ( $17 \times 34$ ) waren 4 triploid und 32 tetraploid, wogegen reziprok ( $34 \times 17$ ) sich von 18 Bastardpflanzen 17 als triploid und 1 Pflanze als tetraploid erwiesen. Das häufige Vorkommen von Tetraploiden in der Richtung der niederchromosomigen Form als Mutterpflanze im Vergleich zu den triploiden Pflanzen versuchten wir in Verbindung mit der Literatur auf die verschiedene Lebensfähigkeit der triploiden und tetraploiden Zygoten zurückzuführen.

Von der in zwei weitgetrennten Verbreitungsgebieten auftretenden Sektion *Leptandra*, deren Verbreitungsverhältnisse HUBER (1929) kartographisch erfaßte, stellte dieselbe Autorin bei *V. virginica* die Chromosomenzahl  $n = 17$  fest.

Eine ganz andere Grundzahl kommt aber der ja auch in allen morphologischen Eigentümlichkeiten wie in ihrer geographischen Verbreitung eine völlige Sonderstellung in der Gattung einnehmenden Sektion *Hebe* zu. Umfassende Untersuchungen wurden hier von SIMONET (1934), durchgeführt, der für *Hebe* die Grundzahl 20 stellte. Wir geben die folgende Übersicht:

- $n = 20$  bei: *V. Gaunteeltii*, *V. Mathewsii*, *V. parviflora*, *V. pimeloides*, *V. salicifolia*, *V. speciosa*.
- $n = 40$ : *V. buxifolia*
- $n = 60$ : *V. Traversii*
- $n = 21$ : *V. Hulkeana*.

Daneben ergab sich nach HUBER (1927) für *V. diosmifolia* die Zahl  $2n = 24$ .

Es tritt also die Grundzahl 20 häufig auf, auf welcher sich dann eine polyploide Reihe aufbaut, Daneben aber kommen offenbar auch abgeleitete Zahlen vor. Man wird, ehe hier weitere Schlüsse gezogen werden können, abwarten müssen, bis noch eine größere Artenzahl dieser umfangreichen Sektion chromosomal durchforscht sein wird.

Natürlich blieb das Ziel meiner Arbeit die zusammenfassende Behandlung der Gattung *Veronica*. Da mich aber selbst so vielerlei Einzelprobleme beschäftigten, so habe ich meinen Schüler RÖMPP (1928) veranlaßt, eine Vorarbeit zu dieser Systematik der Gesamtgattung durchzuführen. Ich glaube, diese Vorarbeit ist so weit-schichtig und glücklich gelungen, daß sie noch auf lange hinaus als Grundlage für vielerlei Untersuchungen innerhalb der Gattung



dienen kann, wie sie als solche für alle Arbeiten im hiesigen Institut seit Erscheinen gedient hat. Mancherlei anatomische Untersuchungen sind seither als Ergänzung durchgeführt worden. Es sei hier nur an die Arbeiten über die Endospermhaustorien der Gattung von GSCHIEDLE (1924) und WEISS (1932) erinnert. Immer weiter aber müssen die systematischen, pflanzengeographischen, histologischen Gesamtergebnisse mit den cytologisch-genetischen in Verbindung gebracht werden. Das wird aber in befriedigender Weise nur auf Grund sehr eingehender, neuer vergleichender Betrachtung möglich sein. Die Feststellung der verschiedenen Grundzahlen und der auf ihnen sich aufbauenden polyploiden Reihen oder abgeleiteten Chromosomenzahlen werden die dauernde Grundlage für all die weiteren Studien abgeben.

#### Literatur

- Bateson, W. u. A. On variation in the floral symmetry of certain plants having irregular corollas. Journ. Linn. Soc. Botany 1891, 28, 386-424.
- Bateson, W. u. D. F. M. Pertz. Notes on the inheritance of variation in the corolla of *Veronica Buxbaumii*. Proc. Cambridge Philos. Soc. 1898, 10, 78-92.
- Beatus, R. Der Erbgang der Pentasepalie bei den Zwischenrassen von *Veronica Tournefortii*. Jahrb. wiss. Bot. 1934, 79, 256-295,
- a) Die *Veronica*-Gruppe Agrestis der Sektion Alsinebe Griseb., ein Beitrag zum Problem der Artbildung. Ztschr. f. ind. Abstgs- u. Vererbungsl. 1936, 71, 153-381.
- b) Genetische und cytologische Untersuchungen in der *Veronica*-Gruppe *Fruticulosa* der Sektion Veronicastrum. Flora 1936, 30, 153-175.
- c) Die Tetradenstruktur der Chromosomen in den prae-metaphasischen Stadien der Meiosis einiger *Veronica*-Arten. Ber. d. D. Bot. Ges. 1936, 54, 340-345.
- d) Polyploidie und Artbildung. Der Biologe 1936, 5, 297-303.
- Correns, C. Lang- und kurzgriffliche Sippen bei *Veronica gentianoides*. Biol. Centralbl. 1924, 43, 610-630.
- Filzer, P. Die Selbststerilität von *Veronica syriaca*. Ztschr. f. ind. Abstgs- u. Vererbungsl. 1926, 42, 137-197.
- Fischer, J. Zur Entwicklungsgeschichte und Morphologie der *Veronica*-Blüte. Ztschr. f. Bot. 1920, 12, 113-160.
- Fries, R. E. Zur Kenntnis der Scrophulariaceen des tropischen Ostafrika. Acta Horti Bergiani 1924, 8, 45-70.
- Graze, H. Die chromosomalen Verhältnisse in der Sektion Pseudolysimachia Koch der Gattung *Veronica*. Jahrb. wiss. Bot. 1932, 77, 507-559.
- Weitere Chromosomenuntersuchungen bei *Veronica*-Arten der Sektion Pseudolysimachia Koch. Jahrb. wiss. Bot. 1935, 81, 609-662.
- Gscheidle, A. Über Haustorienbildung in der Gattung *Veronica* und ihre systematische Wertung. Flora 1924, 17, 144-172.
- Härle, A. Die Arten und Formen der *Veronica*-Sektion Pseudolysimachia Koch etc. Biblioth. Bot. 1932, 104, 86 SS.
- Hagerup, O. Studies on polyploid ecotypes in *Vaccinium Uliginosum* L. Hereditas 1933, 18, 122-128.
- Heitz, E. Der Nachweis der Chromosomen; vergleichende Studien über ihre Zahl, Größe und Form im Pflanzenreich. I. Ztschr. f. Bot. 1925-26, 18, 625-681.
- Hofelich, A. Die Sektion Alsinebe Griseb. der Gattung *Veronica* in ihren chromosomalen Grundlagen. Jahrb. f. wiss. Bot. 1935, 81, 541-572.
- Huber, A. Beiträge zur Klärung verwandtschaftlicher Beziehungen in der Gattung *Veronica*. Jahrb. f. wiss. Bot. 1927, 66, 359-380.



- Huber, A. *Veronica*, die Gruppe *Leptandra* und die Sektionen *Pseudolysimachia* und *Veronicastrum*. Die Pflanzenareale 1929, 2. Reihe, H. 4.
- Juel, H. O. Studies öfver *Veronica* Blomman. Acta Horti Bergiani, 1891, 1, 1-20.
- Lehmann, E. Wanderung und Verbreitung von *Veronica Tournefortii* GM. Abh. nat. Ges. Isis, Dresden 1906, 91-107.
- a) Vorläufige Mitteilung über Aussaatversuche von *Veronicis* der Gruppe *Agrestis*. Ber. d. D. Bot. Ges. 1907, 25, 464-470.
- b) *Veronica agrestis* im Mittelmeergebiet, Ostafrika und Asien. Bull. 1' herb. Boiss. 1907, 2 me sér. VII. S. 546.
- Geschichte und Geographie der *Veronica*-Gruppe *Agrestis*. ibid. 8, 1908, 229.
- a) Einige Mitteilungen zur Kenntnis der Gattung *Veronica*. Österr. Bot. Zeitschr. 1909.
- b) Über Zwischenrassen in der *Veronica*-Gruppe *Agrestis*. Ztschr. f. ind. Abstgs-u. Vererbungsl. 1909, 2, 144-208.
- Über Merkmalseinheiten in der *Veronica*-Sektion *Alsinebe*. Ztschr. f. Bot. 1910, 2, 577-602.
- Über Bastardierungsuntersuchungen in der *Veronica*-Gruppe *Agrestis*. Ztschr. f. ind. Abstgs- u. Vererbungsl. 1914, 13, 88-175.
- *Veronica javanica* BLUME, ein Ubiquist tropischer und subtropischer Gebirge. Annales du Jardin Bot. Buitenzorg 1912, 10, 187-201.
- Vererbungsversuche mit *Veronica syriaca* ROEM. et SCHULT. Ber. d. D. Bot. Ges. 1917, 35, 611-619.
- a) Die Pentasepalie in der Gattung *Veronica* und die Vererbungsweise der pentasepalen Zwischenrassen. Ber. d. D. Ges. 1919, 36, 1. Generalvers.
- b) Über die Selbststerilität von *Veronica syriaca*. Ztschr. f. ind. Abstgs- u. Vererbungsl. 1919, 21, 1-47.
- a) Über die Selbststerilität von *Veronica syriaca* II. ibid. 1921, 27, 161-177.
- b) Über die Vererbungsweise der pentasepalen Zwischenrassen von *Veronica Tournefortii*. Ztschr. f. Bot. 1921, 13, 481-511.
- The heredity of self-sterility in *Veronica syriaca*. Mem. Hortic. Soc. New York 1927, 3, 313-320.
- Geschichte und Geographie der *Veronica*-Gruppe *Megasperma*. Biblioth. Bot. 1929, 29, 55 SS.
- Riek, R. Systematische und pflanzengeographische Untersuchungen in der *Veronica*-Sektion *Chamaedrys* Griseb. Rep. spec. nov. regni veget. Beih. 1935, 79, 68 SS.
- Römpf, H. Die Verwandtschaftsverhältnisse in der Gattung *Veronica*. ibid. Beih. 1928, 50, 172 SS.
- Saunders, E. R. A study of *Veronica* from the viewpoint of certain floral characters. Journ. Linnean Soc. Bot. 1934, 49, 453-493.
- Scheerer, H. Experimentelle und zytologische Untersuchungen innerhalb der *Veronica*-Gruppe *Pentasepala*. Flora, 1937, 31.
- Schlenker, G. Systematische Untersuchungen über die Sektion *Beccabunga* der Gattung *Veronica*. Rep. spec. nov. regni veget. Beih. 1936, 40.
- Experimentelle Untersuchungen in der Sektion *Beccabunga* Griseb. der Gattung *Veronica*. Flora N. F. 1936, 30, 305-350.
- Simonet, M. Contribution à l'étude caryologique des *Veronica*. C.R. des séances de la Soc. de biologie 1934, 117, 1153-1156.
- Tischler, G. Die Bedeutung der Polyploidie für die Verbreitung der Angiospermen. Bot. Jahrb. 1935, 67, 1-35.
- Watzl, B. *Veronica prostrata* L., *Veronica Teucrium* L. und *Veronica austriaca* L. Abh. Zool. Bot. Ges. Wien 1910, 5, 1-94.
- Weiss, E. Weitere Beiträge zur Kenntnis der Endospermhaustorien in der Gattung *Veronica*. Flora N. F. 1932, 26, 418-464.
- Wulff, E. Les *Véroniques* de la Crimée et du Caucase et leur rôle dans l'histoire de la flore du Caucase. 1914, 179 SS.
- Zamelis, A. u. A. Melderis: Pseudogamie bei der selbststerilen *Veronica pinnata* L. infolge der Bestäubung mit dem Pollen von *Veronica longifolia* L. Acta Horti Bot. Univ. Latviensis 1935, 6, 159-191.

**Studies on the Male Gametophyte in Angiosperms, II. Differentiation and behaviour of the vegetative and generative elements in the pollen grains of *Crinum*<sup>1)</sup>**

By

**Nobuhide Suita**

Tokyo Imperial University

(With one plate and 16 text-figures)

According to the majority of the recent investigators, the transformation of the vegetative nucleus in the course of the microspore development is a sort of degeneration or disintegration (4, 8, 10, 11, 12, 13, etc.). The question whether the vegetative nucleus plays a part in the development of the pollen tube, however, still remains unsettled for lack of experimental studies (cf. 8, 10, 13, 17). The question of the existence, origin and nature of the cytoplasmic sheath around the generative nucleus has long been discussed by many investigators, but most of them studied only with fixed materials. It is quite necessary, however, to make observations on both living and fixed materials for the study of this sort of problem.

The present paper deals with the problems of the transformation of the vegetative nucleus and the origin and nature of the cytoplasmic sheath in certain species of *Crinum* which have been investigated in the living or fixed state.

**Materials and methods**

*Crinum asiaticum*, L. var. *japonicum*, Bak., *C. latifolium*, L., *C. gigas*, Nakai, *C. lineare*, L. fil. and two other forms were used in the present study, of which the first two were the most frequently employed. Anthers of *C. asiaticum*, var. *japonicum* were collected and studied in the Misaki Marine Biological Station of the Tokyo Imperial University, while those of the other species were collected from those plants cultivated in the Koisikawa Botanic Garden of the Tokyo Imperial University.

Living materials were observed in the following manner: pollen grains, together with the mucilage, are taken out of the anther and quickly put into a drop of liquid paraffin on a hollow slide and gently covered. When it was necessary, the preparations were kept during period of observation in a moist atmosphere under the microscope. Sometimes glycerin or 1-2% sugar solution was also employed.

1) Contributions from the Divisions of Plant-Morphology and of Genetics, Botanical Institute, Faculty of Science, Tokyo Imperial University, No. 186.

For the fixation and staining of the pollen grains, the acetocarmine method was most suitable. But the paraffin section method and the smear method were also employed to compare the staining reactions of the generative and vegetative elements. They were fixed in Kaiser's, Lenoir's or chrom-acetic solutions and stained with haematoxylin or gentian violet.

To apply Feulgen's nucleal-reaction, materials were preliminarily fixed in Kaiser's solution (corrosive sublimate and acetic acid in water) or the "nucleal-fixative" (corrosive sublimate 5 gr. and acetic acid 3 c.c. in 30% alcohol 150 c.c.), both of which gave satisfactory results. The fixed materials were hydrolysed in 1 N hydrochloric acid for 5–15 minutes at 60°C and immersed in fuchsin-sulphurous acid for 2–3 hours, and washed in sulphurous acid, then in running water.

All the photomicrographs (plate 34) were taken by a simple camera devised by the writer. Special techniques will be described later in each case.

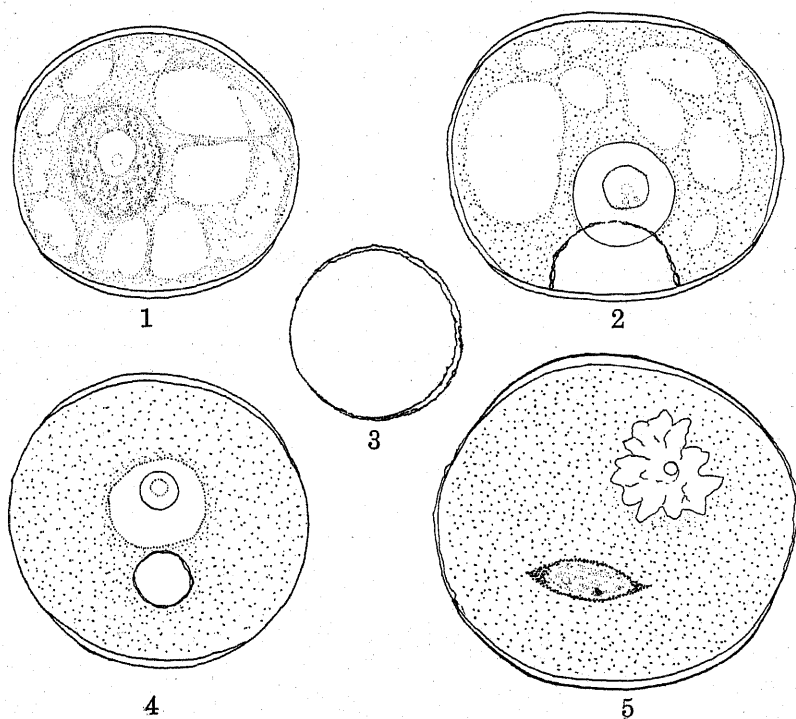
### General description of the microspore development

The walls of the pollen tetrad are formed successively and the arrangement of four cells in each tetrad is of various types. At a later stage, the one-nucleate young microspores separate from each other and become independent cells in the anther mucilage. The young microspore has a characteristically sculptured exine, the orientation of which gives a definite external form to the microspore (pollen grain). The long axis of the pollen grain agrees with the direction across the centres of both sides of the exine. The long diameter of the cell is 30–40  $\mu$  at this stage when measured in acetocarmine preparations. In the course of the development described above, the enlargement of the pollen grain is accompanied by an increase of the nuclear substance, but not of the cytoplasm to the same extent, consequently the cell becomes vacuolate at this stage (text-fig. 1).

The primary pollen nucleus then occupies a definite position, i.e. the central region of one half of the pollen grain. When the pollen grain reaches 45–50  $\mu$  in diameter, the nucleus begins to divide there, its axis being either perpendicular to the long axis or along the short axis of the grain. As to the nuclear division in the pollen grain, two facts are noted: 1) Divisions in pollen grains of one and the same anther occur not simultaneously but quite irregularly, the feature being contrary to that in the case of the reduction division in pollen mother cells. 2) The direction of the division is almost invariable and the spindle is always oriented along the short axis of the pollen

grain in the normal condition. But this polarity can be disturbed by artificial treatments, on which the writer will report in future.

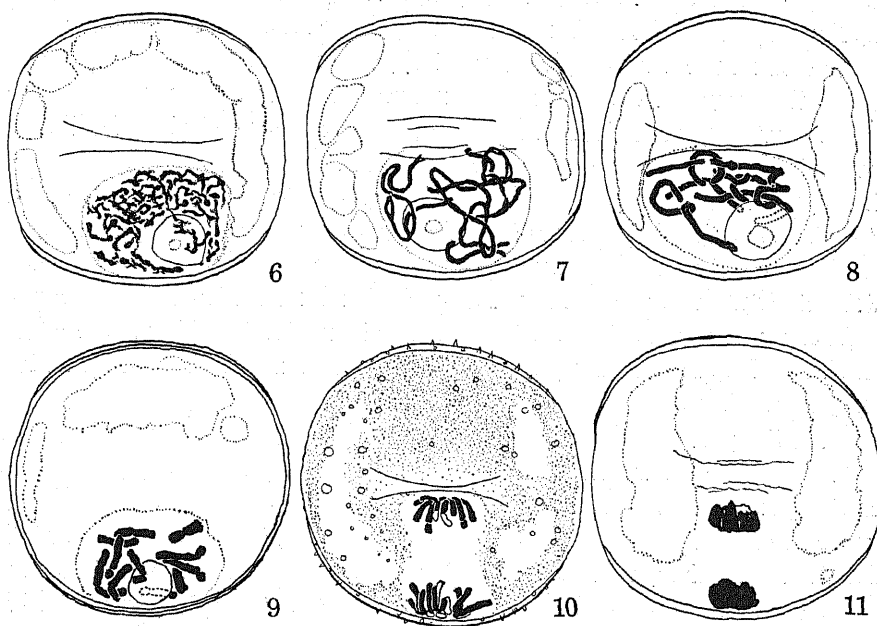
The course of this nuclear division is almost the same as that which takes place in other somatic cells. In the prophase, the nucleus is enlarged to occupy the half of the cell, and contains a large nucleolus throughout this stage, which often remains until the metaphase begins (text-fig. 9). As the division takes place in the half of the cell, one end of the spindle comes in contact with the intine and the other lies free in the cytosome. In this stage, the cell tends to become vacuolate in a marked degree, with occasional formation of a few large vacuoles. In extreme cases, two large vacuoles develop on both sides of the spindle and a cytoplasmic cylinder which involves the mitotic figure is left in the middle of the cell (pl. 34, fig. 6). These vacuoles, however, disappear sooner or later after the nuclear



Figs. 1-5. Pollen development observed in living materials of *Crinum latifolium*. 1. One-nucleate young pollen grain.  $\times 800$ . 2. Young pollen grain after the primary microspore division. Granules in vegetative cytosome, still in Brownian motion. Partition wall surrounding generative cell, somewhat granular.  $\times 800$ . 3. Base of partition wall, where it touches intine. Observed on the direction through short axis of pollen grain.  $\times 1330$ . 4. Pollen grain in a later stage, where generative cell separates from intine and vegetative nucleus begins to be transformed. In this figure, generative cell is drawn in its transverse section.  $\times 660$ . 5. Mature pollen grain.  $\times 600$ . 1-4. in liquid paraffin; 5. in 2% sugar solution.

division. Entering into the anaphase, the daughter chromosomes diverge to the opposite poles of the spindle, and become crowded together in a compact mass. The chromosome-group closing up to the intine may sometimes remain somewhat loose so as to appear wider than the other group lying free in the cytosome (text-fig. 10), but the total actual volume of the chromosomes in each group seems to be nearly equal. An unequal distribution of the chromatic substance between the two daughter nuclei as inferred by Geitler (6) does not seem to have taken place in the course of the nuclear division in *Crinum*.

In the telophase, the formation of the partition wall is evident, though a detailed observation on the process of its development has not yet been carried out. At the later stage, the partition wall comes in contact with the intine and encloses the daughter nucleus formed near the intine, including no granular cytoplasm but a small amount



Figs. 6-11. The primary nuclear division in pollen grains of *Crinum latifolium*, dealt with aceto-carmin. Details are omitted except fig. 10.  $\times$ ca. 800.

of hyaloplasm (probably the phragmoplasm) as seen in text-fig. 12. Throughout the course of the division, from the prophase to the telophase, many lipid-like drops appear in the cytosome of the cell (text-fig. 10).

After the cytokinesis in the pollen grain has been finished, or previous to the passing of the nuclei into the resting stage in extreme

cases, a marked differentiation begins to take place between the two daughter nuclei. The nucleus formed near the intine becomes contracted and retains an intense staining-power, while the other nucleus becomes enlarged, taking a certain liquid into itself and rapidly begins to lose its chromaticity. The former becomes the generative nucleus which divides in the pollen tube to form two male nuclei, and the latter the inactive pollen tube nucleus or vegetative nucleus. The generative nucleus, being like a convex lens in form, lies close to the intine and is enclosed by the thin membrane to give rise to the generative cell including a small amount of hyaloplasm. This condition persists for a considerable length of time. The vegetative nucleus increases more and more in size by the intake of a certain liquid, and at the same time the chromatic substance which shows a stronger refraction-power in living materials gradually diminishes and finally disappears. At the same time, the nucleus increases in size and becomes vacuolate. At this stage, the vegetative nucleus is a large hyaline sphere of lower refractability, involving a large vacuolate nucleolus in the centre (pl. 34, fig. 10), and no longer distinguishable from the vegetative cytosome by means of its staining reaction for aceto-carmin, though it is quite easily distinguishable in living materials owing to the violent Brownian motion of the granules in the cytosome. The proportion of the diameters of the pollen grain (in this case the short diameter), the vegetative nucleus, the nucleolus of the vegetative nucleus and the generative nucleus is approximately 7:3.5:1.5:1. This condition is reached in 20-30 hours after the primary microspore division and persists for a considerable length of time. The size of the pollen grain has now reached 55-60  $\mu$  in its longer diameter.

As the pollen grains develop and increase in size, the anther loses its mucilage and becomes gradually dessicated, and the cytoplasm of the pollen grains gradually becomes gelatinized. When the pollen grain reaches 70-75  $\mu$  in diameter, the generative cell separates from the intine of the pollen grain and becomes a little elongated, while the vegetative nucleus begins to become transformed (text-fig. 4). The pollen grain which has come to maturity is 80-85  $\mu$  in its long diameter when measured in aceto-carmin, and is full of coarse granules which are probably reserve materials. The generative nucleus in the mature pollen grain is ellipsoid and surrounded by the cytoplasmic sheath, thus forming the spindle shaped generative cell (text-fig. 5). A cataphoretic experiment shows the electric charge of the exine of the mature pollen grain to be negative in a sugar solution of pH 7 or thereabouts.

The haploid chromosome number is counted as 11 in the primary

microspore division in *C. asiaticum*, var. *japonicum*, *C. gigas* and *C. latifolium*. The diploid number is 22 in the root tip cells of these species.

### Degeneration of the vegetative nucleus

As described in the foregoing, the vegetative nucleus begins to increase greatly in size soon after the primary microspore division. This enlargement takes place generally in the resting stage, accompanying the scattering of the granular masses of chromatin as well as the diminution of the staining-capacity of the nucleus as a whole. In some cases, such loosening of the chromosome-mass begins so early in the telophase that the resting stage is omitted in the course of this transformation. In such cases, the chromatic strings are loosely distributed in the nuclear sap, which seems to show a partial dissolution of the strings. This fact seems to indicate that the enlargement of the vegetative nucleus has been caused not only by a strong swelling of the nuclear colloid, but also by other physical and chemical changes of the nuclear substance.

In a later stage, where the chromatic substance has entirely disappeared and the nucleus has increased greatly in size, a membrane-like layer draws a distinct line between the vegetative cytosome and the vegetative nucleus. This state of the nucleus is compared with a drop of oil lying in water. This nucleus, a hyaline sphere of a lower refractability, is indistinguishable from the cytosome from the point of view of staining-capacity with aceto-carmin, showing that the nucleus may have no "chromatic substance" yet. Being fixed in chrom-acetic acid and stained with haematoxylin, it shows itself as a vague sphere stained only a little more intensely than the cytosome (pl. 34, fig. 1). By Feulgen's nucleal-reaction it is homogeneously stained a weak reddish-violet as the writer already reported (15). The intensity of this staining seems to diminish gradually as the pollen develops. These facts may allow the conclusion that the thymus nucleic acid contained in the chromosomes has dissolved and diffused homogeneously into the nuclear sap and that the dissolved thymus nucleic acid (or its decomposition-product) is gradually decomposed into other compounds from which no aldose can be obtained by hydrolysis.

It is to be questioned how such a change has taken place only in the vegetative nucleus, while not in the generative nucleus. This phenomenon may reasonably be ascribed to a chain of fermentative actions. The ferments, such as protease and nuclease, will possibly dissolve the nuclear substance when the vegetative nucleus stands under conditions permitting the action of the ferments. The



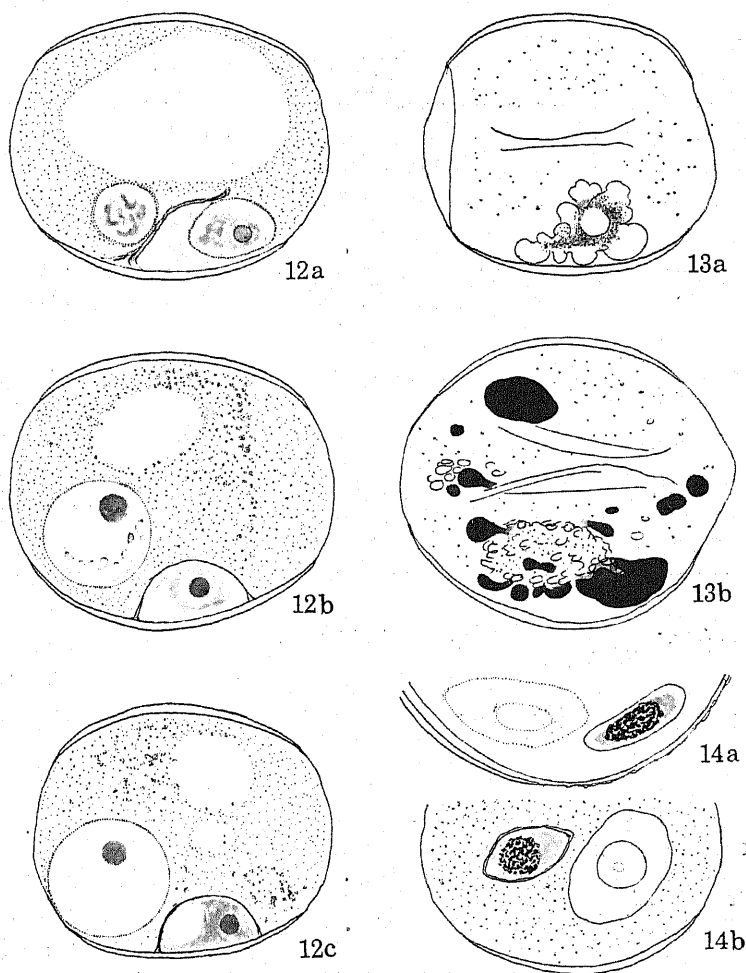
possibility of the existence (or production) of such ferments in the pollen grain has been experimentally proved in the species of *Crinum* concerned. The experiment has been carried on as follows: a young inflorescence was cut from the main flower-stalk at the joint and kept in a moist and air-tight chamber for 6-7 days. The microspores remained alive for 3-4 days in this condition. After 6-7 days, all the nuclei, the primary pollen-nucleus and the vegetative and the generative nuclei, became decomposed in various degrees. One nucleus decomposed and partially changed to lipoid-substances, another nucleus nearly disappeared and another entirely disappeared, so that the pollen-cells came to contain a number of lipoid-drops which were intensely stained by Sudan III (text-fig. 13). These remnants of the nuclear elements were no longer positive to Feulgen's reaction. Such changes can be ascribed only to fermental actions. Although these changes are not strictly the same as those of the vegetative nuclei in the course of the normal development of the pollen grain, it is not incredible that the above mentioned ferments are present from the beginning or newly produced in the pollen grains of *Crinum*-species.

While the changes in the vegetative nucleus seem due to fermentative actions, there remains the fundamental question as to why the generative nucleus has been left undecomposed. The visible differences between the conditions of both nuclei can be seen at first in the conditions of the two poles of the spindle and then in the behaviour of the cell-plate in the primary microspore division, as well as in the nature of the cytoplasm which comes in contact with each nucleus. These differences seem to have brought about abnormal conditions only to the vegetative nucleus but not to the generative nucleus.

Probably by the process of gradual dehydration, the enlarged vegetative nucleus is transformed at first as seen in text-fig. 14, and then into an amoeboid shape at the stage of the pollen-maturation. Accordingly, its volume gradually diminishes in the process of the transformation, accompanying the greater diminution of the volume of the nucleolus. The amoeboid nucleus is still hyaline, and indistinguishable from the cytosome by the aceto-carmin method. Being fixed in the solution of picric acid and formalin and stained with haematoxylin, it is still amoeboid in form and is stained homogeneously and somewhat darker than the cytosome (pl. 34, fig. 2). It is stained only so faintly by Feulgen's method that it is difficult to determine its definite form by this method (pl. 34, fig. 4). This degree of intensity of the staining persists almost invariably, although staining seems to be a little stronger in some cases, up till the time

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the generative nucleus divides in the pollen tube to form two male nuclei, or later on. It shows that the chemical component of the vegetative nucleus, thymus nucleic acid or its decomposition-product, which can be hydrolysed into aldose and other compounds, has changed to certain other compounds in the course of the pollen development, but a small amount of it has been left unchanged throughout the life of the microgametophyte. The amoeboid shape of the nucleus in a mature pollen grain cannot be easily changed by immersing the



Figs. 12-14. 12. Successive observation of cytokinesis in living pollen grain of *Crinum latifolium*. The material was in an abnormal condition, which brought an irregularity to the direction of the division. a. Partition wall is formed.  $\times 960$ . b. One hour later.  $\times 960$ . c. 18 hours later.  $\times 880$ . 13. Artificial decomposition of nuclear substance in young pollen grains of *Crinum latifolium*. Descriptions in text. a. Dealt with aceto-carmin. b. Dealt with Sudan III.  $\times 800$ . 14. Generative cell, which became separated from intine.  $\times \text{ca. } 800$ .

pollen grain in 2% sugar solution or 50% acetic acid. But in a strong electric current, the nucleus diffuses away and disappears except for the nucleolus, while the generative nucleus becomes coagulated, retaining its characteristic shape.

These facts seem to indicate that the enlargement and hyalinization of the vegetative nucleus are not only due to simple swelling of the nucleus but also to the chemical decomposition and irreversible dissolution which accompanies further physical changes. This is possibly an abnormal change for a nucleus, and may be designated as "degeneration". It is questionable, however, whether or not such a "degenerate" nucleus plays any part in the life of the pollen tube. A definite conclusion cannot be given until critical experiments are conducted on this problem.

### The generative nucleus and its cytoplasmic sheath

After the primary microspore division, the generative cell is enclosed by a thin membrane, which is at first apparently thin and uniform in structure, but, in a later stage, it seems to become somewhat granular. The membrane forms a circle at the base where it touches the intine of the pollen cell, which is much thicker on one

side than on the other (text-fig. 3). Being treated with 45% acetic acid (in aceto-carmine), the membrane swells in a marked degree as shown in text-fig. 15.

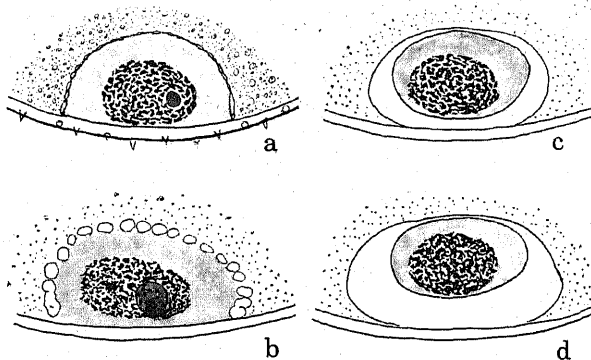


Fig. 15. Apparent swelling and fusion of partition wall treated with aceto-carmine. a. Immediately after the treatment. b. One hour after the treatment. c, d. Granular structures fused to form a homogeneous wall which gradually swells from c to d in 30 minutes.  $\times 1330$ .

That the cytoplasm in the generative cell is hyaline and contains no granules, is evidently recognizable not only in the course of the

cytokinesis but also in the state of the complete cell. Considering the features of the cytokinesis as shown in text-fig. 12, the generative cytoplasm appears to have been derived from the phragmoplast.

In a mature pollen grain, the generative nucleus is surrounded by the characteristic sheath of cytoplasm and the substantial membrane is no longer present around the cell. This sheath contains a

number of lipid-like granules which are distributed in a spindle-shape in the hyaloplasmic medium, but still contains no fine-granules such as are found in the vegetative cytosome. These granules are seen lightly yellowish in living materials and seem to be lipid-droplets though they have not shown a distinct positive reaction to Sudan III in the present experiment. They are entirely negative to Feulgen's nucleal-reaction. Under the action of acetic acid these granules swell and fuse, so that the cytoplasmic sheath becomes enlarged and apparently homogeneous (text-fig. 16).

It is an interesting question as to what source these lipid-like granules have been derived from. In the early stages of the pollen development, the generative nucleus is surrounded by the hyaline cytosome and the thin membrane. This condition lasts perhaps until the

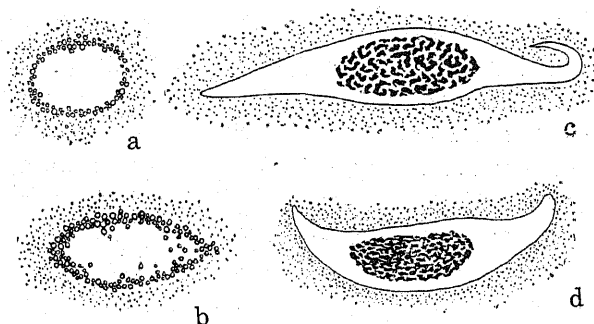


Fig. 16. Generative cell in mature pollen grain of *Crinum latifolium*. a, b, in living state; a, transverse section, b, side view. c, d. Dealt with aceto-carmin. Lipoid-like granules are fused and become apparently expanded.  $\times 1330$ .

generative cell separates from the intine, but at this stage the membrane has already shown a granular structure and seems to be a chain of granules (text-fig. 2). In a later stage, the membrane disappears and lipid-like granules appear. A detailed observation failed to disclose the transition between these stages, but the lipid-like granules are supposed to have been derived from the material of the membrane or the hyaline cytoplasm. As regards the reaction to acetic acid, there is a close resemblance between the lipid-like granules and the generative cell membrane. The origin of these granules, however, cannot be concluded by such a simple resemblance. The apparent expansion of these elements may be ascribed to the shrinkage of the cytoplasm. On the other hand, it is possible that lipid-substances segregate from the ground substance of the hyaline cytoplasm, the origin of which is supposed to be in the phragmoplast (Shinke and Shigenaga, 1933, proved that the phragmoplast contained lipid). Be the matter what it may, such a granules containing structure is believed to be a normal state in the completed cytoplasmic sheath, and not an artifact.

The generative nucleus is invariably stained intensely with ordinary staining-agents as well as by Feulgen's reaction throughout the course of the development (pl. 34, figs. 1-4). In a mature pollen grain, the generative nucleus seems to be in a prophase-like condition. The nucleolus of the generative nucleus of the mature pollen grain is difficult to recognize, though its existence is clearly evident in the telophase of the primary microspore division (text-fig. 15; pl. 34, fig. 7).

### Discussion and conclusion

The changes in the vegetative nucleus in the course of the pollen development are quite remarkable and entirely different from the state during the resting stage. It can be considered as an abnormal condition for a nucleus. These changes, at least in the investigated species of *Crinum*, are not caused by a normal action of the cytoplasm resulting in an increase of the nuclear substance, but by a decomposing action on the chromatic substance in company with other related phenomena. These abnormal changes, especially the remarkable decrease of thymus nucleic acid which plays an important part in the process of respiration in the cell, are responsible for the assumption that the vegetative nucleus has become a degenerate element of lower vital activities as stated by the writer in the previous paper (15).

In a recent paper, Geitler puts forward an assumption that the differentiation between the vegetative nucleus and the generative nucleus depends on the quantitative difference between the cytoplasm surrounding each nucleus, namely, on the quantitative difference in the cytoplasmic actions. It is obvious, however, that Geitler's assumption cannot be applied to the case of *Crinum*, when the above mentioned nature of the change in the vegetative nucleus is considered, together with the qualitative difference between the vegetative cytoplasm and the generative cytoplasm. The differentiation between these two nuclei may be due to both quantitative and qualitative differences in the surrounding conditions. One of these differences may be seen in the differential activity of ferments, i.e. the ferments decompose the substance of the vegetative nucleus while they are inactive in the generative nucleus. Such a remarkable change of the vegetative nucleus seems to imply "degeneration" of the nucleus rather than to imply positive acquirement of vital activity. Poddubnaja-Arnoldi (1936) described experiments on the nuclear-pollen-tubular relation by X-ray treatment which seemed to lead to the conclusion that no nuclei were needed for the life of the pollen tube. This conclusion seems somewhat incredible. On the other

hand, it may be probable as Wulff (1933) suggested that the vegetative nucleus plays a part such as the production of ferments in the pollen tube. But concerning the problem as to whether or not it plays a part in the life of the pollen-tube, no definite conclusions can be drawn until exact experimental studies have been carried out.

According to the recent investigators, the presence of the cytoplasmic sheath surrounding the generative nucleus is evident in many species of angiosperms (2, 3, 4, 5, 7, 9, 17, 18, etc.), including *Crinum* in the present case. Although the nature of the sheath is not the same in all the species, most of them appear to be easily broken by the action of improper fixatives. Some of the descriptions that the nucleus was naked might have been based on such artifacts arising in fixation. The generative cell and the sperm cells of every species must be investigated not only in fixed materials but also in living materials.

### Summary

1) The investigations of the pollen development in *Crinum*-species are reported with special reference to the behaviour of the vegetative nucleus and of the generative cell in their living or fixed state.

2) The primary microspore division occurs invariably along the short diameter of the pollen grain, one end of the spindle being free in the cytosome and the other end close to the intine. The daughter nucleus formed on the free end of the spindle becomes the enlarged and inactive vegetative nucleus, while the nucleus formed close to the intine becomes the generative nucleus and is enclosed by a thin wall to form the generative cell. When the pollen comes to maturity, the vegetative nucleus, being gradually dehydrated, becomes amoeboid in form, while the generative cell is transformed into a spindle-shape and lies free in the vegetative cytosome.

3) The vegetative nucleus begins to increase in size and to lose its chromaticity soon after the primary microspore division. About one day after the division, the nucleus becomes a homogeneously hyaline sphere and is only faintly stained by Feulgen's method. The chromatic substance in the vegetative nucleus seems to have dissolved and diffused homogeneously into the nuclear sap. In the mature pollen grain, the nucleus is amoeboid in shape and is stained by Feulgen's method less than in the above mentioned state, showing a remarkable decrease of thymus nucleic acid. Considering the features mentioned above, the vegetative nucleus is supposed to be a degenerate element.

4) The generative nucleus is intensely stained by ordinary

staining methods as well as by Feulgen's at every stage of the pollen development. In the mature pollen grain the nucleus often appears to be in a prophase-like condition, being surrounded by the characteristic sheath of cytoplasm which consists of a hyaline medium and many lipoid-like granules. The origin of these constituents of the generative cytosome has not yet been ascertained.

5) The haploid chromosome number is 11 and the diploid number is 22 in all three plant forms: *C. asiaticum*, var. *japonicum*, *C. gigas* and *C. latifolium*.

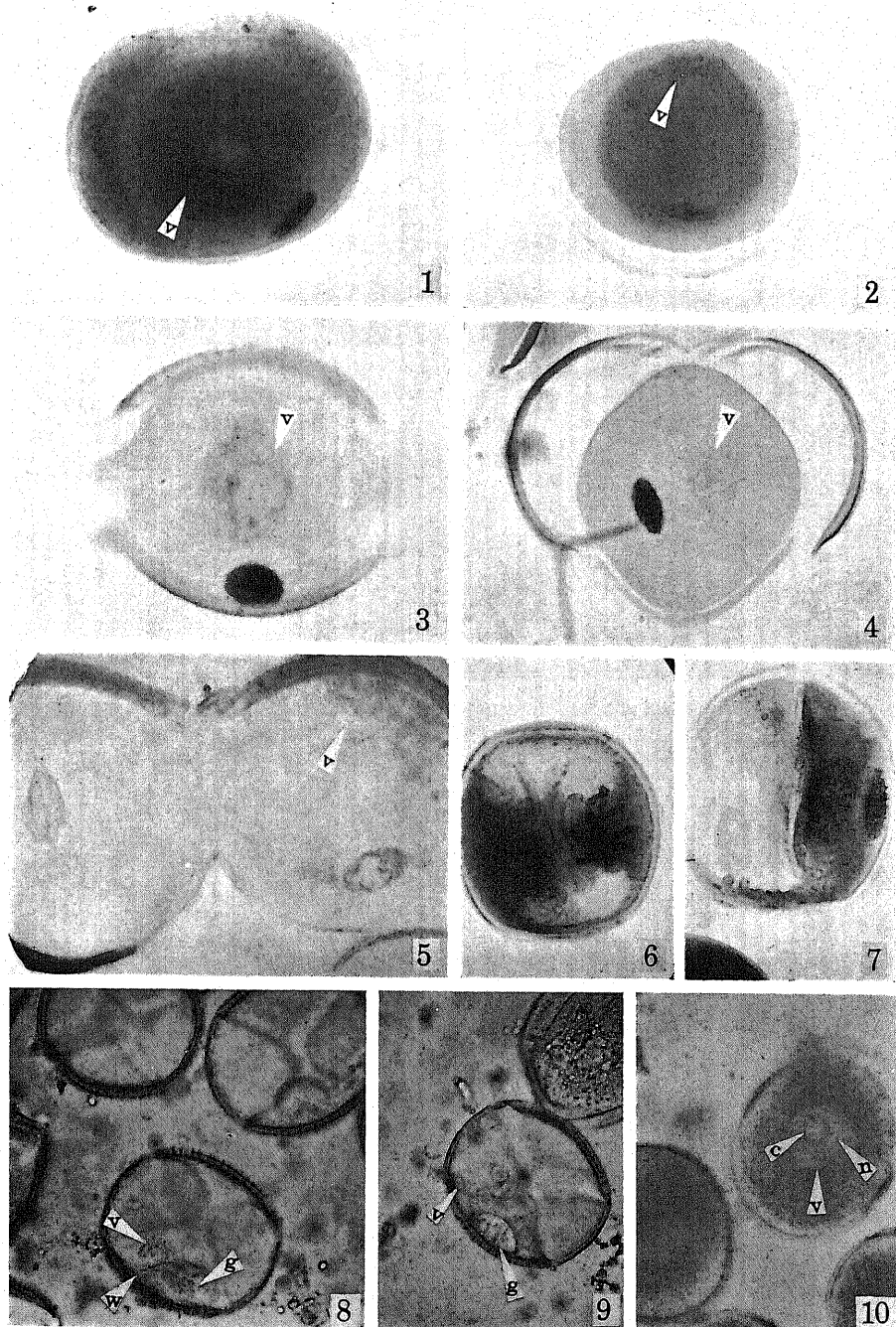
The writer wishes to express his cordial thanks to Dr. Sinotô for his helpful suggestions and criticism throughout the course of this investigation.

Division of Genetics, Botanical Institute,  
Faculty of Science, Tokyo Imp. University

#### Literature

- 1) Billings, F. H. 1934. Male gametophyte of *Atriplex hymenelytra*. Bot. Gaz., 95: 477-484.
- 2) Cooper, D. C. 1935. Microsporogenesis and the development of the male gametophytes in *Portulaca oleracea*. Amer. Journ. Bot., 22: 453-459.
- 3) — 1936. Development of the male gametes of *Lilium*. Bot. Gaz., 98: 169-178.
- 4) Cooper, G. O. 1935. Cytological studies in the Chenopodiaceae. Bot. Gaz., 97: 167-178.
- 5) Finn, W. W. 1935. Einige Bemerkungen über den männlichen Gametophyten der Angiospermen. Ber. Deut. Bot. Ges., 53: 679-686.
- 6) Geitler, L. 1935. Beobachtungen über die erste Teilung in Pollenkorn der Angiospermen. Planta, 24: 361-386.
- 7) Locke, J. F. 1936. Microsporogenesis and cytokinesis in *Asimina triloba*. Bot. Gaz., 98: 159-168.
- 8) Poddubnaja-Arnoldi, W. A. 1933. Künstliche Kultur und zytologische Untersuchung des Pollenschlauches von *Senecio platinaefolius* BENTH. Planta, 19: 299-304.
- 9) — 1933. Spermazellen in der Familie der Dipsacaceae. ib., 21: 381-386.
- 10) — 1936. Beobachtungen über die Keimung des Pollens einiger Pflanzen auf künstlichen Nährboden. ib., 25: 502-529.
- 11) Sax, K. 1935. The effect of temperature on nuclear differentiation in microspore development. Journ. Arn. Arb., 16: 301-310.
- 12) — 1936. The experimental production of polyploidy. ib., 17: 153-159.
- 13) — and Edmonds, H. W. 1933. Development of the male gametophyte in *Tradescantia*. Bot. Gaz., 95: 156-163.
- 14) Shinke, N. and Shigenaga, M. 1933. A histochemical study of plant nuclei in rest and mitosis. Cytologia, 4: 189-221.
- 15) Sua, N. 1936. Studies on the male gametophyte in angiosperms, I. Studies in the degenerating process of the pollen tube nucleus, with special reference to Feulgen's reaction. (Japan., with Engl. summary). Botany and Zoology (Tokyo), 4: 2033-2042.
- 16) Transkowsky, D. A. 1930. Zytologische Beobachtungen über die Entwicklung der Pollenschläuche einiger Angiospermen. Planta, 12: 1-18.
- 17) Wulff, H. D. 1933. Beiträge zur Kenntnis des männlichen Gametophyten der Angiospermen. Planta, 21: 12-50.
- 18) — 1934. Lebendefärbung mit Chrysoidin. ib., 22: 70-79.





Suita: Studies on the Male Gametophyte in Angiosperms, II.



## Explanation of plate 34

The photomicrographs were taken with Agfa-Isochrom plates and a Wratten B 2 filter. All the materials are the pollen grains of *Crinum latifolium*. g, generative nucleus and cell; v, vegetative nucleus; n, nucleolus; c, vacuole; w, partition wall.

- Fig. 1. Young pollen grain, where generative nucleus has just separated from intine. Fixed in chrom-acetic acid and stained with iron alum-haematoxylin.  $\times 770$ .
- Fig. 2. Mature pollen grain, fixed with a mixture of picric acid and formalin and stained with haematoxylin.  $\times 650$ .
- Fig. 3. Young pollen grain after the primary microspore division, stained by Feulgen's method.  $\times 770$ .
- Fig. 4. Mature pollen grain, stained by Feulgen's method.  $\times 770$ .
- Fig. 5. Mature pollen grains in living state.  $\times 540$ .
- Figs. 6-7. The primary microspore division. 6. Side view of metaphase. 7. Formation of two daughter nuclei. Dealt with aceto-carmin.  $\times 550$ .
- Figs. 8-9. Cytokinesis of the primary microspore division. Drawn at text-fig. 12, a and b.  $\times 450$ .
- Fig. 10. Young pollen grain, considerably long after the primary microspore division.  $\times 450$ .

## The Behaviour of the Plastid as a Hereditary Unit : The Theory of the Plastogene

By

Yoshitaka Imai

Faculty of Agriculture, Tokyo Imperial University

### Introduction

Since plastid originates from plastid by division, a plastid has its own individuality. The characteristics of the plastid are usually controlled by the genotype, or gene complex, by mutation of which a variant character appears and is transmitted as a Mendelian unit. The variant characteristics of the plastid do not depend upon its own trait, so that the property of the plastid, or plasmotype (IMAI 1936b), is normal. Generally speaking, the plasmotype of the plastid is highly constant, as is usually the case with the gene. The plastid, however, spontaneously changes its plasmotype by mutation, which process is called plastid mutation. The mutant plastid may propagate by repeated division and distributes into cells. For example, white mutant plastids form in this way white tissues or patches in the leaves and other chlorophyllous organs, presenting variegation. Such a plastid character is inherited as non-Mendelian.

Analogous to the mutable gene, the plastid sometimes mutates with recurrent frequency. I have divided this case into two classes, auto-mutation and exo-mutation, the former mutating by itself and the latter through the effect of a certain gene (IMAI 1928, 1934b, 1936a). In my opinion, certain variegated forms of *Oryza sativa* studied by Japanese investigators are the result of recurrent auto-mutation of plastids. In *Oryza*, plastid mutation is either from unstable green to stable white and stable green, or from unstable green to stable white. In *Hordeum vulgare*, an automutable yellowish plastid appeared by sporadic mutation from normal (IMAI 1936c). In my view (IMAI 1936a), WINGE's variegated form of *Humulus japonica* may be regarded as due to recurrent automutation of green and white plastids. Presumably, in this case, both green and white plastids are mutable to each other. For exomutable plastids, we can cite a number of cases (IMAI 1928, 1934a, 1936a, 1936b), which are grouped under two classes. In the one the plastid mutates from green to stable white, and in the other to unstable white. In stable white, the white variegation extended sometimes to cause the bud variation to be pure white, and the variegated plants gave albino

seedlings, although in some cases, either none or only a few such seedlings resulted through zygotic lethality of the albino embryos. In unstable white, the white variegation has green ticks or lobes, from which originate some green seedlings in the offspring. A large part of the chlorophyll variegation, according to my examination, are due to recurrent exomutation of plastids. Rarely, the mutated plastids do not revert to the prototypic green, but to yellow.

### The Plastid and the Plastogene

Since in inheritance, a plastid behaves as a unit, and the mutant plastid maintains its new characteristic from cell to cell and from generation to generation without change or modification, unless new plastid mutation occurs, the plastid has something that is heritable or a factor that manifests the characteristic. A new term "plastogene" is given to this factor. The character of the plastid is manifested by the plastogene under the control of the gene complex. Generally the plastogene is highly constant, and sporadically changes its plasmotype, giving a mutant plastid. The change in the plastogene of the plastid is due to mutation of the plastogene. In the mutable plastids, the plastogenes are unstable and plastid mutation occurs recurrently in one or more directions. Although we cannot see the plastogene under the microscope, it is supposed that plastid division is preceded by division of the plastogene. Each plastid contains one plastogene, which, divides and enters the daughter plastids after plastid division. Since a plastid contains only one plastogene, the division is simple, that is direct. The plasmotype is also simple, no heterozygous condition of plastogenes occurs, but some cells may have mixed plastids of different plasmotypes, seeing that a cell contains a number of plastids in its cytoplasm. The nucleus is a receptacle for the chromosomes, which are strings of a number of chromomeres. Each chromomere, therefore, is divided at nuclear division in order that the chromosomes may be allocated into equal parts. Mitosis is the total figure of direct divisions of the respective chromomeres.

In bacteria, no nucleus is formed, only scattered chromatins being found. The blue-green algae (Schizophyceae) are a bridge to the more complicated organisms in the evolution of chromatin structure by exhibiting an "incipient nucleus". The chromatin found in the Schizophyta is regarded as a primitive chromosome, probably an aggregate of a certain number of chromomeres. In this connection, I shall make bold to say that the plastid is developed from the common body together with the chromatin. If this is admitted, the

unit of chromosome, or chromomere, corresponds to the plastid and the gene to the plastogene. In the course of evolution, some of the common bodies formed chromatins, enclosed in a nucleus, while others developed into plastids, left in the cytoplasm. Under the microscope, the plastid found in a mature cell is relatively a large body, whereas the proplastid found in a meristematic cell is very small in size, so that the plastid undergoes marked growth in its life history. The plastid may be built by a "nucleus" plastogene and the covering matrix plastophore. The growth in size is due to development of the plastophore, which contains pigments in the coloured plastids. In the salivary gland cells of some insects are found giant chromosomes, with lengths as much as from about 100 to 150 times that of the oogonia chromosomes, which indicates the possibility that the matrix of the chromosome swells up greatly in certain media. The relatively large body of the plastids, therefore, does not seem out of reason.

The theory of the plastogene advanced above applies well to the complex behaviour of the plastid mentioned in the beginning of this paper. In the following pages, further observations on the chlorophyll variegation of some plants are described in connection with the application of this theory.

### *Tropaeolum majus*

Variegation in *Tropaeolum majus* is transmitted as simple recessive to green (RASMUSON 1920; CORRENS 1920; BATESON, cited from MOFFETT 1936), the character being regarded as a "type" in inheritance. In my opinion, however, the variegation is not so simple in its manifestation, the mechanism lying in the recurrent mutation of plastids. The exomutation of plastids from green to greenish yellow is accompanied by recurrent reverse automutation of plastids. The exomutation is conditioned by a recessive gene, which produces variegation by bringing about plastogene mutation. The green tissues that occur in the yellowish mesophyll are usually small in size, forming green ticks, so that automutation of the yellowish plastogene seems to take place generally at late cell generation in the ontogeny of the leaf. The yellowish tissues may cover an entire leaf or even a branch, although usually remain to form the mottling in the leaf. Rarely, variegated plants were observed to bear all greenish yellow leaves in the course of development. The yellowish parts have invariably green ticks.

The variegated breeds true to type, giving uniform offspring. The seedlings have cotyledons, which are variegated very slightly. The variegation is not apparent in the early developed leaves, it being gradually marked in the later leaves: the fourth or fifth leaf is fully



marked in the matter of variegation, presenting fine mottling (Fig. 1). Pollen and ovules are quite fertile and the production of seeds per capsule is also normal, as shown in Table 1.

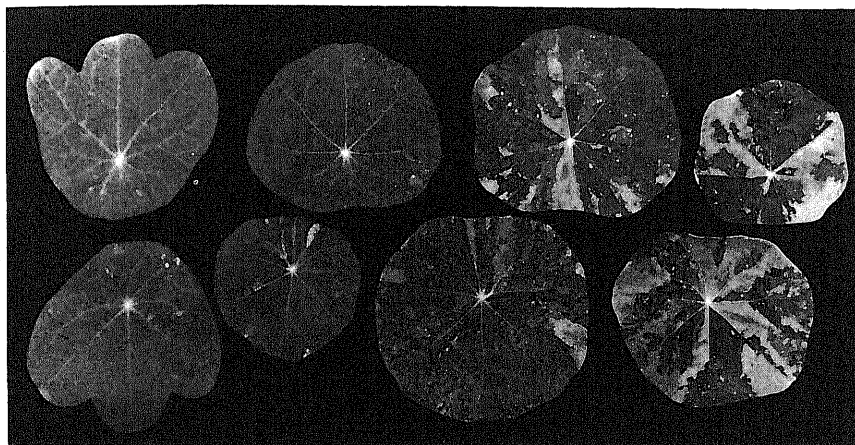


Fig. 1. Cotyledons (left two) and early developed leaves of the variegated *Tropaeolum*, taken from a seedling and arranged in the order of their development.

The germination rate is also substantially the same, showing 92 per cent in the variegated and 93 per cent in normal. These experiments were conducted in a greenhouse. The results gave negative proofs for the lethality of the yellowish gametes and zygotes. In these circumstances, I think that variegation is reduced to the pseudo-green condition at gametogenesis. At this stage, the exomutation

Table 1. Seed production in *Tropaeolum*

Character of plant	Seed number per capsule			Total of seeds	Average seeds per capsule
	1	2	3		
Variegated	29	44	43	246	2.12
Green	16	27	24	142	2.12

of the plastogene from green to yellowish seems to stop, and the yellowish plastids mutate with high frequency to green as the result of their automutability. There is nothing strange in this, seeing that plastid mutation is greatly influenced by environments, especially by the condition of the cell.

The absence of recurrent mutation at gametogenesis has been already pointed out in *Hordeum* and *Oryza* in connection with exomutation in the former and automutation in the latter. Non-mutability at gametogenesis in *Tropaeolum* is therefore not peculiar



to this plant alone. The phenomenon of "retroversion" is observed also in the case of pseudo-mutation, as pointed out by IMAI and KANNA (1927) in *Amaranthus*.

With the theory of retroversion of the character at gametogenesis, the problem of non-production of the yellowish seedlings is solved. Since retroversion in this case is partly conditioned by recurrent automutation of the yellowish plastids, should they be unable to bring about automutation to green, we ought to expect some yellowish seedlings in the offspring. In the offspring of the white-variegated *Hordeum*, white seedlings appear in a fairly definite proportion, while no albino seedlings are produced in the progeny of the variegated form of *Polygonum orientale*. In both cases, variegation is the result of recessive genes, which change the plasmotype of the plastids from green to white, or in other words, exomutation of the plastogene takes place recurrently. In *Polygonum*, albino embryos, however, fail to germinate because of zygotic lethality. Owing to non-automutability of the chlorophyll deficient plastids to green, retroversion of the character at gametogenesis is hindered, so that the result is albino embryos after fertilization. In the variegated *Pelargonium*, automutation of the plastids from albinotic to green is exhibited in some cases, in which small green ticks occur at times in the creamish tissues (IMAI 1936b). The albino seedlings partially germinated in my experiments. Automutability, however, is not frequent in *Pelargonium*, so that retroversion of the character is not much in evidence, giving albino seedlings. Retroversion therefore seems to be completely accomplished in variegated forms, in which the frequency in the automutation of the plastogene is high.

The embryos of the variegated *Tropaeolum* start from pseudo-green, and by recurrent exomutation of the green plastids, aided by the automutation of yellow plastids, the cotyledons and leaves are variegated. The frequency of exomutation, which is not high during early zygotic development, is increased later, although the extent to which it is done is greatly influenced by environments. The mechanism involved in the variegation of *Tropaeolum* is the same as that shown elsewhere (IMAI 1936a, Figure 7B).

The alternation of yellowish plastids to green is regarded as being due to automutation of the plastogene, and not to exomutation. Since the variant yellowish plastid is conditioned by the change in the plasmotype, its property is already beyond the control of the gene complex, whence mutation should occur automatically. In *Pelargonium*, white plastids with automutability are also held in the green plants, when the plastids are introduced by crossing, which proves that alternation of chlorophyll deficient plastids is not due to the

gene that stimulates exomutation of plastids, but to the property of the plastogene itself, that is, automutation.

***Polygonum virginianum* var. *filiforme***

The variegated form of *Polygonum virginianum* var. *filiforme* has creamish white patches in the leaves, and green ticks occur in the creamish tissues (Fig. 2). The mechanism by which variegation occurs is the same as that of the variegated *Tropaeolum*: the creamish patches are due to recurrent exomutation of the plastogene from green to creamish, and the green ticks to the recurrent automutation of the plastogene from creamish to green.



Fig. 2. Variegated form of *Polygonum virginianum* var. *filiforme*.

The variegated gave only 218 variegated seedlings, no albinos being observed. Retroversion at gametogenesis in connection with variegation seems to be also complete in this case. The seedlings generally start from apparently green, variegation appearing gradually in the leaves. Since the shoots from old stocks also are nearly green when young, retroversion seems to occur also at the meristematic cells of discontinuous growth. However, in 1935, one of the old variegated stocks produced permanent green branches, which, on selfing, revealed their heterozygosity by giving seedlings that segregated for green and variegated, judging from which the

variegated form of *Polygonum* seems to tend to revert to the green condition by gene mutation. Mutation here takes place from the gene "variegated", which alters green plastids to creamish ones, to its normal allele.

### *Pharbitis Nil*

The variegated form (Fig. 3) of the Japanese morning glory, *Pharbitis Nil*, is inherited as simple recessive, when the variegation is considered as a whole. The variegated breeds true to type, neither white nor green offspring being found. The cotyledons are sometimes



Fig. 3. Variegated leaf of *Pharbitis Nil*.  
(Leaf form: dragonfly retracted).

variegated, and a few of the early leaves are usually slightly variegated. Variegation gradually becomes apparent with growth, at the end of which, however, the small ill-developed leaves assume heavy variegation.

The range in the degree of variegation is considerable, including even green and white leaves. The green leaves, although very rare, may occur at the axillary branches. When a plant is pinched, the newly developed vigorous branches usually bear slightly variegated leaves by recovering its juvenescent growth. In Figure 4 are shown four leaves which exhibit variation in the extent of variegation, including a pure green.

The production of green leaves is due to fluctuation. The white leaves, which also are very rare in occurrence, have usually green ticks. Figure 5 shows white and variegated leaves, the latter of which has large green-ticked white sectors.

In my opinion, variegation of this plant is not simple, being conditioned by recurrent exomutation of green plastids and recurrent automutation of white plastids. Through mutual changes, white variegation with green ticks occurs. Since manifestation of white variegation is due to the exomutation of plastids from green to white, the chances for mutation are greatly controlled by environments. Roughly speaking, rich growth lessens its frequency, while

poor growth increases it. In this plant, green-ticked white leaves are only rarely observed, and the white branchlets very rarely, notwithstanding that white variegation is so common that almost every



Fig. 4. Leaves picked from a variegated plant of *Pharbitis*, exhibiting fluctuations in the extent of variegation, including a green leaf. (Leaf form: dragonfly).

leaf is mottled with white tissues. This limitation means that the exomutation of plastid does not usually occur at the growing points of the stems and leaves. Since the Japanese morning glory is a



Fig. 5. Leaves of *Pharbitis*; the left two having large white sectors, the right one being white. Note small green ticks in the white parts. (Leaf form: maple).

trihistogenic plant, white-over-green chimeras may be expected. In the variegated leaves, white patches occur side by side, some in the outer tissues, some in the inner tissues, and others in both tissues. In Figure 3, for example, the dark parts have green mesophyll, the light parts white outer and green inner tissues, and the white parts

white mesophyll. In the leaves that have developed at the end of the growth, however, we found some to be white-over-green peri-



Fig. 6. Periclinal (left) and mosaic (right) leaves of *Pharbitis*. (Leaf form : dragonfly).

clinal, although they are frequently found with more or less non-periclinal parts (Fig. 6). A close observation of leaves of variegated plants shows these periclinal leaves, although not frequent—



Fig. 7. Variegated-reduced leaf of *Pharbitis*. (Leaf form : normal).

a fact strongly supporting the view that white variegation is not a simple "type" as formerly supposed, but is conditioned by recurrent exomutation of the plastogene, induced by a recessive gene "variegated". The white leaves or white parts of leaves have green ticks, which are regarded as the result of recurrent

automutation of the plastogene. Thus, variegation is conditioned by two factors of reversible plastid mutations. The fact that the green tissues form generally small ticks indicates that the automutation occurs late in the cell generation of the leaf.

Under these circumstances, we should expect white seedlings among the variegated offspring, but my experience during the last twenty years denies it. The pollen and ovules are quite fertile as in normal green, no lethal gametes having been found. Neither zygotic lethality is detected; the average proportion of seeds per capsule is substantially alike for green and variegated plants; the germination rate also does not differ. These observations force me to conclude that the absence of white seedlings is due to retroversion of the character at gametogenesis, whence the mechanism involved in *Pharbitis* is exactly the same as that in *Tropaeolum*.

For variegation in *Pharbitis*, a modifier, variegated-reduced was found (IMAI 1925), by means of which very finely mottled variegation (Fig. 7) occurs with the aid of the gene "variegated". In the leaves, recurrent automutation of plastogene from whitish to green occurs very frequently during late cell generation of the leaves, forming fine green speckles in the white tissues. The gene variegated-reduced modifies the rate of mutation and the time at which mutation occurs. With this exception, the mechanism of variegation is the same as that of ordinary variegation of this plant.

### *Polygonum Blumei*

A variegated form of *Polygonum Blumei* exhibits white mottling in the leaves. In the leaves of the variegated plant, a wide range of variegation occurs from pure green to almost white with green ticks (Fig. 8). Although the variation is concerned with the extent of variegation, it is confined to rather limited ranges. The significance in its variation is in the distribution of variegation, in other words, the degree of mosaic of the green and white parts. Roughly speaking, the leaves or the sectors of the leaves are grouped under two classes, green (genetically pseudo-green) and variegated. Generally the borders of the mosaics are abrupt. The variegated form gives graded offspring according to the extent of variegation, as shown in Table 2.

Besides variegated individuals, the offspring included apparently green ones which, however, were unstable. These plants, when cultivated with ample space will show variegation in their leaves. They, however, give equally variegated offspring as well. In the variegated offspring, a series graded with respect to the amount



of alternate distribution of green and variegated parts occurred, including whites with green ticks, which, however, grew poorly owing to the smaller amount of photosynthesis.

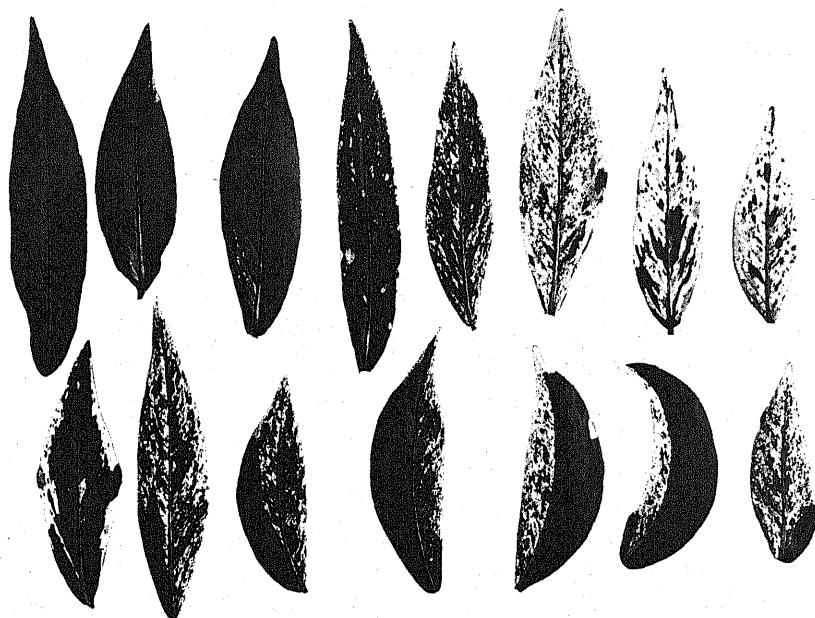


Fig. 8. Variation in the variegated pedigree of *Polygonum Blumei*, including mosaics.

The variegated individuals frequently bear mosaic leaves and branches, and green and variegated branches occur as bud variation.

Table 2. Offspring of the variegated *Polygonum*

Plant or branch	Pseudo-green or almost green	Slightly variegated	Variegated	Heavily variegated	Total
Pseudo-green	20 18.87%	66 62.26%	16 15.09%	4 3.77%	106
Slightly variegated	4 7.27%	38 69.09%	9 16.36%	4 7.27%	55
Variegated	56 24.78%	120 53.10%	40 17.70%	10 4.42%	226
Heavily variegated	14 10.14%	83 60.14%	29 21.01%	12 8.70%	138

A separate observation of the offsprings from the green and variegated branches of the same plant are given in Table 3.

Although the results show some differences, they do not seem important. As to the mechanism of variegation in this plant, my opinion is as follows: Since crossing experiments have not been



made, it cannot be decided whether alternation of the plastogene involved in the present case is due to automutation or to exomuta-

Table 3. Offspring of green and variegated branches

Branch	Pseudo-green or almost green	Slightly variegated	Variegated	Heavily variegated	Total
Pseudo-green	18 25.00%	43 59.72%	7 9.72%	4 5.56%	72
Heavily variegated	2 5.13%	24 61.54%	11 28.21%	2 5.13%	39

tion. Most probably, there are three plastogenes to account for the mechanism of variegation, namely,

1. Green-alpha: a plastogene is responsible for the green plastid, and having relatively low mutability, produces pseudo-green leaves.
2. Green-beta: a plastogene is responsible for the green plastid, and having high mutability, produces white leaves with green ticks.
3. White: a plastogene is responsible for the white plastid, and having high mutability, produces white leaves with green ticks.

As stated above, it is not clear whether the cause of the change is due to the property of the plastogene itself (automutation) or to a special gene (exomutation). These plastogenes, however, seem to mutate reversibly with respect to each other, resulting in variation in the variegated pedigree, sometimes also exhibiting mosaics. The reversible mutation is influenced considerably by environments. Owing to the characteristics and the mutability of the respective plastogenes, three kinds of leaves result. The pseudo-green leaves have plastids containing green-alpha, which may change into either green-beta or white, the former mutation resulting in green and variegated mosaics and the latter in green and white (with green ticks) mosaics. The variegated leaves have plastids of both green and white as the result of high reverse mutation. The plastogene green-beta may also transform to green-alpha, by which mutation variegated and green mosaics are produced.

The young seedlings are generally green or slightly variegated, showing that they start from the pseudo-green condition. In this case, retroversion of the character in connection with variegation seems to occur also at gametogenesis, due to absence of mutation from pseudo-green to variegated or white and to their frequent reverse mutation. Consequently, no albino or heavily variegated seedlings

occur, and the heavily variegated plants or branches give substantially the same offspring as the pseudo-green ones.

In *Polygonum Blumei*, no periclinal leaves occur. I examined the species of *Polygonum*, together with *P. orientale* and *P. virginianum* var. *filiforme*, but no periclinals for chlorophyll deficiency were observed. In *Tropaeolum*, the absence of periclinal leaves was also noted, so that among dicotyledons, these species may be regarded as dihistogenic plants.

### Conclusion

Plastid mutation is regarded as a change in the plastogene contained in the plastid. The plastid propagates by direct division, preceded, presumably, by division of the plastogene. A plastid may correspond to the chromomere that is located in the chromosome. The mechanism of some chlorophyll variegations lies in the recurrent auto- and exomutation of the plastogenes. Through plastogene mutation, variant plastids appear among otherwise homogeneous plastids of a cell, variegation being exhibited when they propagate into the cells. Anatomical examination of the variegated leaves reveals the fact that some cells contain mixed plastids of different plasmotypes.

In the variegated forms, in which the rate of automutation of the plastid from white (or yellowish) to green is high, retroversion of the character in connection with variegation takes place at gametogenesis, as the result of which no albino seedlings appear. All zygotes start from an apparently green condition, variegation gradually appearing in the leaves of the seedlings. Retroversion is conditioned by hindrance of plastogene mutation from green to white and by increase in the frequency of its reverse mutation. Therefore the rate of plastogene mutation is greatly influenced by the condition of the cell. In meristematic cells, plastogene mutation is frequently hindered. Generally speaking, rich nourishment or rejuvenescence of tissues decreases the rate of such mutation.

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### Literature Cited

- Correns, C. 1920. Vererbungsversuche mit buntblättrigen Sippen. III-V. Sitzungsber. d. Preuss. Acad. Wiss. 6: 212-240.  
Imai, Y. 1925. Inheritance of deformed leaves in *Pharbitis Nil*. Bot. Gazette 80: 276-287.  
— 1928. A consideration of variegation. Genetics 13: 544-562.  
— 1934a. An apparently simple inheritance of variegation in *Polygonum orientale*. Jour. Genetics 29: 147-151.

- Imai, Y. 1934b. On the mutable genes of *Pharbitis*, with special reference to their bearing on the mechanism of bud-variation. Jour. Coll. Agri., Tokyo Imp. Univ. 12: 479-523.
- 1936a. Chlorophyll variegations due to mutable genes and plastids. Zeitschr. f. ind. Abst.- u. Vererbgs. 71: 61-83.
- 1936b. Geno- and plasmotypes of variegated pelargoniums. Jour. Genetics 33: 169-195.
- 1936c. Recurrent auto- and exomutation of plastids resulting in tricolored variegation of *Hordeum vulgare*. Genetics 21: 752-757.
- and Kanna, B. 1927. On the variability of a white-eared form in *Amaranthus paniculatus*. Genetics 12: 242-252.
- Moffett, A. A. 1936. The genetics of *Tropaeolum majus*. Jour. Genetics 33: 151-161.
- Rasmuson, H. 1920. Die Hauptergebnisse von einigen genetischen Versuchen mit verschiedenen Formen von *Tropaeolum*, *Clarkia* and *Impatiens*. Hereditas 1: 270-276.
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## Zur Frage der serologischen Differenzierbarkeit biologischer Rassen

Von

O. Moritz und H. L. Rohn

### Einleitung

Die Verwendung serologischer Methoden für die Zwecke der biologischen Verwandtschaftsforschung geht auf UHLENHUTH (1905) und NUTTALL (1904) zurück. Die Übertragung vom zoologischen Gebiet in das Gebiet der Botanik geschah durch MAGNUS and FRIEDENTHAL (1907), sowie durch die großangelegten Arbeiten MEZ' und seiner Schüler. Als allgemein anerkanntes Ergebnis der serologischen Verwandtschaftsforschung kann man den Satz aufstellen, daß nahe verwandte Arten Gleichheiten hinsichtlich ihrer serologisch reaktionsfähigen Eiweißkörper aufweisen, während im allgemeinen systematisch einander sehr fernstehende Arten keinerlei serologisch faßbare Eiweißgemeinschaft aufweisen (Ausnahmen: Eiweiß der Augenlinse der Tiere). Als problematisch muß auch heute noch die Frage gelten, in welchem Maße es gelingt, Eiweißgemeinschaften bei verwandten Pflanzenfamilien einer systematischen Reihe oder gar bei verschiedenen Reihen nachzuweisen. Die im hiesigen Institut erfolgten Nachuntersuchungen des MEZschen Stammbaumes ergaben jedenfalls bislang nur in sehr begrenztem Umfange derartige Eiweißgemeinschaften (MORITZ: 1929 u. 1934a). Ein weiteres Problem ist die Frage, bis zu welchem Grade es gelingt, mit serologischen Methoden bei sehr nahe verwandten Arten oder gar Rassen Eiweißverschiedenheiten zu entdecken. Nach den bisherigen Ergebnissen der Arbeiten im hiesigen Institut versagt die serologische Differenzierung schon häufig beim Versuch der Artunterscheidung, ja der Unterscheidung von Gattungen. So konnten z.B. bislang Eiweißunterschiede zwischen *Raphanus* und *Brassica* nicht festgestellt werden (MORITZ: 1934b). Auch in vielen anderen Fällen versagte die Methode noch, obgleich andererseits manche Unterschiede genau erkannt und beschrieben werden konnten, so bei den KIHARASchen *Aegilops-Triticum*-Bastarden, bei amphidiploiden *Roggen-Weizen*-Bastarden (MORITZ: 1934b), bei einem zweifelhaften Bastard zwischen Linse und Wicke (MORITZ u. v. BERG: 1931).

Immerhin lag es nahe, nach den erwähnten Mißerfolgen bei der Rassen- u. Artdifferenzierung auf botanischem Gebiet, nunmehr

Material zu untersuchen, das von anderen Forschern anscheinend mit Erfolg für serologische Rassendifferenzierung verwendet worden war: Seren verschiedener menschlicher Rassen. Den experimentellen Teil der Arbeit leistete dabei der eine von uns (R.), die theoretische Auswertung versuchte der andere (M.).

### Experimenteller Teil

Als Ausgangspunkt unserer Untersuchung wählten wir die im Jahre 1907 erschienene Arbeit von C. BRUCK. Anlässlich der NEISSERschen Syphilis-Expedition nach Batavia untersuchte BRUCK mittels der Komplementbindungsmethode die ihm dort zur Verfügung stehenden Affenseren und die Seren der dort vorhandenen menschlichen Rassen (Holländer, Javanen, Chinesen und Malayen). Vorerst sei nun ein kurzer Überblick über die BRUCKsche Arbeit und ihre Resultate, sowie über den Mechanismus der von BRUCK verwendeten Komplementbindungsmethode gegeben. Denn zur Verfolgung der vom Verfasser angestellten Versuche ist ihre Kenntnis notwendig.

Bestimmte Immunitätsreaktionen, zu denen auch die spezifische Hämolyse gehört, bedürfen zu ihrem Zustandekommen außer homologem Antikörper und Antigen noch eines eigentümlichen, in frischem Normalserum enthaltenen Stoffes, der als Komplement bezeichnet wird.

Setzt man einem gegen Hammelblutkörperchen gerichteten Kaninchenimmenserum (Ambozeptor) das homologe Antigen (Hammelblutkörperchen) hinzu, so wird keine Hämolyse auftreten, wenn das Komplement im Ambozeptor-Serum zerstört wurde (durch Erhitzen auf 56 Grad). Das Gemisch stellt nun ein Reagenz auf Komplement dar. Für andere Immunitätsreaktionen ist das Komplement nun zwar nicht nötig; vorhandenes Komplement wird aber gebunden, wenn eine spezifische Reaktion zwischen Antigen und Antikörper eintritt. Man kann also mit Hilfe der Ambozeptor-Blutkörperchen-Mischung feststellen, ob in einem anderen Gemisch von Antigen und Antikörper, dem man Komplement zugefügt hatte, eine Reaktion eintrat oder nicht. Im ersten Falle kommt es nicht oder nur zur schwachen Hämolyse. Im zweiten Falle wird das unverbrauchte Komplement bei geeigneten Mengenverhältnissen zur vollkommenen Hämolyse führen.

Auf Grund der UHLENHUTHschen Untersuchungen—UHLENHUTH hat sowohl mit der Präzipitation, als auch mit der Komplementbildung (bei kreuzweiser Immunisierung) sehr feine Differenzen erhalten—entschloß sich BRUCK, stets unter genauester Berücksichtigung der quantitativen Verhältnisse, vorerst festzustellen, ob Eiweißunterschiede innerhalb der Individuen einer Unterart (Rasse) z.B.

*Orang-Utan* oder *Macacus cynomolgus* beständen. Dieses wäre der Fall gewesen, wenn bei einem Anti-*Orang*-Kaninchen-Serum z.B. das eine *Orang*-Serum bis zum Endtiter 1:900, das andere bis zum Endtiter 1:1000 und das dritte bis zum Endtiter 1:700 Hämolyssehemmung ergeben hätten. So wären nach BRUCK individuelle Eiweißunterschiede bewiesen und eine Differenzierung der Rassen infolgedessen sehr in Frage gestellt worden.

Maßgebend für die Auswertung dieser Ergebnisse ist also der sogenannte „Titer“, bis zu welchem die Antiseren mit verschiedenen Antigenen reagieren. Im allgemeinen gilt die Regel, daß das homologe Antigen (das zur Injektion bei der Herstellung der Antiseren benutzt wurde) in stärkster Verdünnung mit dem Antiserum reagiert. Der Titer der Reaktion wird also an der Stärke der Verdünnung des Antigens abgelesen.

Um nun mit den Worten BRUCK's zu sprechen: „Stets war der Titer der Hämolyssehemmung bei allen Tieren derselben Art gleich; ein mit *Orang*-Serum erzieltetes Kaninchenimmenserum wirkte auf alle *Orang*-Seren gleich stark“. Es zeigte sich also die komplette Hämolyssehemmung bei allen *Orang*-Seren bei denselben Verdünnungsgraden (Titern) ebenso bei allen *Macacus cynomolgus*-Seren.

Nach dieser Feststellung untersuchte BRUCK, ehe er an das Problem der Differenzierung menschlicher Rassen heranging, mehrere Affenarten (nicht Unterarten). Es gelang ihm zu zeigen, daß diese untereinander Differenzen aufwiesen. Interessant und zu beachten ist hier, daß mit einem Anti-*Orang*-Serum wohl die übrigen einzeln unterschiedlich reagieren, aber die menschlichen Rassen (Holländer, Chinesen und Malayen) alle bis zum gleichen Titer Hämolyssehemmung zeigen, also unter sich hierbei nicht zu unterscheiden sind. Worauf dieses nach Ansicht BRUCK's beruht, wird noch am Schlüsse der Ausführungen über seine Arbeit erwähnt. Nach dieser Differenzierung ging BRUCK dann an die Unterscheidung der menschlichen Rassen, allerdings unter dem steten Hinweis, daß sich für diese Versuche keine zu hochwertigen Seren eignen. Seine Untersuchungsergebnisse faßte BRUCK in der nachfolgenden Tabelle 1 zusammen.

Aus dieser Tabelle ist nun ohne weiteres ersichtlich, daß bei einem gegen Holländerserum gerichteten System 1. All Individuen der gleichen Rasse bis zum gleichen Endtiter Hämolyssehemmung geben, 2. die verschiedenen Rassen bezüglich des Endtiters gegeneinander unterschieden sind. BRUCK kommt nun zu dem den Ergebnissen nach berechtigten Schluß, daß ein Unterschied des Blutserums der verschiedenen Rassen und in Verbindung damit eine Differenz zwischen den Rassen aufgezeigt ist. Hinsichtlich der Natur dieses Unterschiedes, meint BRUCK: Es scheine danach also eine Rasse zu

Tabelle 1. Der Ergebnisse der Rassendifferenzierungsversuche nach BRUCK.

Kaninchen vorbehandelt mit Holländer-Serum. + = Hämolysehemmung,  
0 = komplette Hämolyse.

0,1 Antiholb.- Serum gepr. m.	Holländer.				Araber	Chinesen.				Malayen.				Orang- utan	Macacus cyno- molg.
	I	II	III	IV		I	II	III	IV	I	II	III	IV		
1:2000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1:1000	+	+	+	+	0	0	0	0	0	0	0	0	0	0	0
1: 900	+	+	+	+	+	0	0	0	0	0	0	0	0	0	0
1: 800	+	+	+	+	+	0	0	0	0	0	0	0	0	0	0
1: 700	+	+	+	+	+	+	+	+	+	0	0	0	0	0	0
1: 600	+	+	+	+	+	+	+	+	+	0	0	0	0	0	0
1: 500	+	+	+	+	+	+	+	+	+	+	+	+	+	0	0
1: 400	+	+	+	+	+	+	+	+	+	+	+	+	+	0	0
1: 300	+	+	+	+	+	+	+	+	+	+	+	+	+	0	0
1: 200	+	+	+	+	+	+	+	+	+	+	+	+	+	0	0
1: 100	+	+	+	+	+	+	+	+	+	+	+	+	+	0	0
1: 50	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0

geben, die alle Partialrezeptoren der übrigen, aber außerdem noch eigene besitzt. Der Holländer besitzt also alle Rezeptoren der Araber, Javanen, Chinesen und Malayen und außerdem noch einige eigene; der Araber besitzt alle Rezeptoren des Chinesen, Javanen, Malayen und eigene, aber nicht die dem Holländer eigenen u.s.w. Beim Ausgang von anderen Zentren aus ergab sich für BRUCK folgendes Bild. Z.B. ausgehend vom Araber zeigten Araber und Holländer bei gleichen Titern Hämolysehemmung, die übrigen stufenweise tiefer, beim Malayenzentrum zeigten alle von ihm untersuchten Seren den gleichen Hemmungsendtiter.

Nach dem Vorliegen dieser Differenzierungen der menschlichen Rassen, war es für BRUCK klar, weshalb er bei der Verwendung eines Affenzentrums keine Differenzierung der Menschenrassen von einander erhalten hatte. Der Affe hat nach ihm nur Eiweißgruppen des dominanten Artrezeptors „Mensch“ mit dem Menschen gemeinsam, aus diesem Grunde mußten bei einem *Orang*-Zentrum alle menschlichen Rassen bis zum gleichen Titer Hämolysehemmung zeigen. Aus diesen Ergebnissen, die ja unzweifelhaft interessant sind und der Wiederholung bzw. der Verifizierung bedürfen, zog nun BRUCK ohne weiteres den Schluß, daß die menschlichen Rassen bezüglich ihres Blut-Serum-Eiweißes qualitativ different wären.

Während BRUCK die Bezeichnung „Rezeptoren“ (Dominanter R. und Partial-R.) verwendet, sind anlässlich der im hiesigen Institut unternommenen serologischen und serodiagnostischen Arbeiten die Bezeichnungen „Protenom“ und „Proten“ geprägt worden (MORITZ + v. BERG 1931). „Protenom“ bezeichnet den Gesamteiweißkomplex, die Gesamtsumme der „Protene“. Als „Protene“ bezeichnen wir die serologisch faßbaren Grundlagen der Artunterschiede.



Bevor die Anstellung der Differenzierungsversuche mittels der Anaphylaxie-Methode in die Wege geleitet wurde, mußte folgende Überlegung angestellt werden: Waren die menschlichen Rassen durch Proteine von einander unterschieden, also ein qualitativer Unterschied vorhanden, dann mußte dieser bei der rein qualitativ arbeitenden Methode des anaphylaktischen Zuckungsversuches eindeutig zu Tage treten. Bezüglich des Wesens der Allergie oder Anaphylaxie sei hier nur auf die einschlägige Literatur verwiesen (z.B. WELLS: 1927).

Vorerst sei nun auch hier auf den Mechanismus der im hiesigen Institut gebräuchlichen und für die nachfolgenden Versuche angewendeten anaphylaktischen Zuckungsmethode eingegangen.

Injiziert man einem Meerschweinchen die Lösung eines artfremden tierischen oder eines pflanzlichen Eiweißes (wir verwenden intraperitoneale Injektion), so bildet der tierische Organismus „Abwehr“-Stoffe, sogenannte Antikörper, gegen jedes einzelne Protein dieses Conglomerates von Einzeleiweißkörpern, des Proteinoms. (Über eventuelle Konkurrenzerscheinungen siehe unten). Sie können beim Meerschweinchen nach einer gewissen Zeitspanne, der sogenannten Inkubationszeit, im glatten Muskelgewebe festgestellt werden, wo sie den allergischen, anaphylaktischen oder sensiblen, überempfindlichen Zustand des Tieres hervorrufen. Im Serum dagegen sind sie beim Meerschweinchen bekanntlich nicht, oder nur schwer nachweisbar.

Injiziert man nämlich nach mehreren Wochen einem solchen sensiblen Tier wiederum dieselbe Eiweißlösung (es genügt schon eine hohe Verdünnung derselben), dann geht das Tier unter den schwersten Krampferscheinungen, dem sogenannten anaphylaktischen Schock, ein. Den Tod des Tieres rufen nun die Kontraktionen der glatten Muskelfaseraggregate hervor; in diesem Falle ist es vor allem die Bronchialmuskulatur, deren Kontraktion den Erstickungstod veranlaßt.

Bei unseren Versuchen benutzen wir nun als glatte Muskel die Uterushörner des Meerschweinchens (DALE'scher Versuch 1912). Man präpariert die Uterushörner eines jungfräulichen, sensibel gemachten Meerschweinchens heraus und hängt sie mit einem Schreibhebel verbunden in die für diese Versuche gebräuchliche Spezialapparatur (MORITZ: 1934a). Wird nun zu dem Bade, in dem sich der isolierte Muskel, also das überlebende Organ des Tieres, befindet, die Lösung des homologen, d.h. des zur Impfung verwendeten Eiweißkörpers, hinzugefügt, dann erfolgt auch hier außerhalb des Organismus die Reaktion durch Kontraktion des Muskels und kann so auf einer mit Rußpapier bespannten, rotierenden Trommel aufgezeichnet werden. Es werden hier, bei Zugabe des homologen Antigens, alle

vorhandenen Antikörper abgesättigt (MORITZ: 1929). Benutzt man zur Zugabe anstatt des homologen Eiweißes, Eiweiß einer verwandten Art, dann werden von den vorhandenen Antikörpern nur die abgesättigt, die für das zugegebene Eiweiß spezifisch sind. Enthält dieses nicht völlig die gleichen Protene wie das homologe, dann wird also erst nach Zugabe des homologen Eiweißes die Absättigung der Restantikörper, die dem Unterschied zwischen dem zuerst zugegebenen und dem homologen Antigen entsprechen, und also erneute Reaktion erfolgen. Hier ist schon zu erkennen, daß man ev. mehrere Differenzierungsreaktionen an einem Muskel vornehmen kann, bis alle Antikörper abgesättigt sind. Es ist also ersichtlich, daß mit dieser Methode etwaige, einigermaßen ausgeprägte qualitative Unterschiede aufgezeigt werden können, daß fernerhin hier eine subjektive Beurteilung der jeweiligen Resultate seitens des Untersuchenden so gut wie ausgeschlossen ist, da ja jede einzelne Kontraktion vermittels des Schreibhebels auf dem Rußpapier aufgezeichnet und nach Fixierung des Papiers mit Schellacklösung jederzeit eingesehen und geprüft werden kann. Das Bild solcher Kurven ist in Abb. 1 bis 4 reproduziert.

Bei Beginn der Injektionszeit, die gewöhnlich 8–14 Tage dauert bei täglicher Dosis von 2 ccm, soll das Gewicht der Tiere nicht unter 150 und nicht über 200 Gramm betragen, da im ersten Falle die Kleinheit der Tiere (die Widerstandsfähigkeit ist noch nicht groß genug) einen verhältnismäßig hohen Tieraussfall bedingt, im zweiten Falle aber die Tiere zur Reaktion zu groß werden und infolgedessen sehr unerwünschte Eigenzuckungen des isolierten Muskels auftreten können.

Von den Ergebnissen BRUCK's ausgehend, mußte man bei vorhandenen Unterschieden annehmen, daß die weiße Rasse sämtliche Protene der übrigen Rassen enthalte. Waren dann noch die anderen Rassen gegeneinander unterschieden, dann war es das Zweckmäßigste, wenn die dem Verfasser zur Verfügung stehenden Seren differenziert werden sollten, die Impfung der Tiere mit „weißem“ Serum vorzunehmen. (Siehe Anmerkung.<sup>1)</sup>)

Es war dann möglich, bei dieser gegen „weiß“ gerichteten Sensibilität an einem Muskel, sowohl den Unterschied zwischen der weißen und irgend einer anderen Rasse, als auch stufenweise nacheinander den Unterschied aller vorhandenen Rassen festzustellen.

Aus diesen Gründen wurden also Immunisationszentren gegen „weißes“ Serum hergestellt und zwar eine Gruppe von 3 Tieren, die

1) Anmerkung: Der Kürze wegen wurden bei den Versuchen und den Aufzeichnungen in der Tabelle für die verschiedenen Rassen nur die entsprechenden Farbbezeichnungen gewählt. z.B. Weiß = Deutschblütig, Schwarz = Neger, Gelb = Japaner, Jude = Jude.

je dreimal mit 2 ccm einer Serum-Verdünnung 0,2:2 geimpft wurden und eine weitere Gruppe von 10 Tieren mit je 4,4 ccm Serum in 14 Dosen à 2 ccm Verdünnung. Diese Gruppe durchlief also eine Injektionszeit von 14 Tagen. Wie bei den botanisch-serologischen Versuchen, so war es auch hier angebracht, während dieser 14 tägigen Injektionsperiode genau Protokoll über den jeweiligen gesundheitlichen Zustand der Tiere und eventuelle Ausfälle zu führen. Im Gegensatz zu den botanischen Versuchen sind bei diesen keine Ausfälle zu verzeichnen gewesen.

Es sei hier gleich eingeschaltet, daß die Aufbewahrung der Seren am zweckmäßigsten auf Eis erfolgt und zwar in Ampullen. Es wurde stets nach der Zentrifugierung des Blutes (die Zentrifugenröhrchen waren vor der jeweiligen Blutentnahme mit Stopfen bei cca 180 Grad eine halbe Stunde lang trocken sterilisiert) das Serum mit einer sterilen Injektionsspritze in sterile Ampullen zu je 2 ccm Fassungsvermögen umgefüllt und nach dem Verschmelzen der Ampullen im Eisschrank aufbewahrt. So hatte man stets die gebräuchliche Menge zur Hand und das Serum hielt sich mehrere Wochen und Monate.

Die Erhöhung der Impfdosis der letzten Gruppe erfolgte aus dem schon oben beiläufig erwähnten Gesichtspunkt der Konkurrenzerscheinungen heraus (MORITZ: 1934b). Es besteht nämlich die Möglichkeit, daß Antigene, die in geringer Menge neben anderen quantitativ reichlich vorhandenen, injiziert werden, gar keine Antikörperbildung veranlassen. Wenn also, wie wohl vorauszusehen ist, die unterscheidenden Protene nur in geringer Quantität neben den gemeinsamen vorhanden sind, so würde unter Umständen ihre Gegenwart dennoch nicht im serologischen Versuch entdeckt werden können. Diese Erscheinung bezeichnet man als „Konkurrenz der Antigene“. Wir wissen nun, daß man diesen Fehler in gewissem Maße ausschalten kann (MORITZ: 1934a u. 1934b), indem man 1. die Injektionsdosen und—Häufigkeit bis zur Grenze des Möglichen steigert, 2. außerdem noch die Inkubationszeit, gerechnet vom Tage der letzten Injektion an, möglichst weitgehend variiert, d.h. zum Beispiel bei einer Gruppe von 10 Tieren nach cca 3–4 Wochen alle 8 oder 14 Tage ein Tier in Reaktion nimmt, beim Auftreten der höchsten Sensibilität dann aber alle Tiere direkt nacheinander bearbeitet.

Hier wurden die Tiere in den verschiedensten Zeiträumen zur Reaktion gebracht. Die Resultate sind aus der folgenden Tabelle 2 ersichtlich. Als Beispiel und zur Erläuterung des Reaktionsverlaufes seien nun einige Kurven reproduziert und kurz auf sie eingegangen.

Betrachten wir zunächst den Verlauf der Kurve des Uterushornes a des Tieres 39 Abb. 1. Bei I wurden 2 ccm 0,2:10 jüdisches Serum in das Bad gebracht. Es erfolgte eine starke Kontraktion des Muskels,

Tabelle 2

## System Anti-Weiß

Laufende Nr.	1.	2.	3.	4.	5.	6.	7.	8.	9.
Tier-Nr.	14.	17.	19.	10.	12.	27.	39.	40.	41.
Injektions- impfungsgruppe	II	II	II	I	I	II	II	II	I
Inkubations- zeit i. Tagen.	15	23	30	36	43	45	70	71	123
Horn	a b	a b	a b	a b	a b	a b	a b	a b	a b
Reaktionen mit 2 ccm Seren- verdünnung 0.2:10	I	Sch- warz +	Gelb +	Gelb +	Gelb +	Gelb +	Gelb +	Sch- warz +	Jude warz +
	II	Sch- warz -	Gelb -	Gelb -	Gelb -	Gelb -	Gelb -	Sch- warz -	Jude warz -
	III	Weiß -	Weiß -	Weiß -	Weiß -	Gelb -	Jude -	Gelb -	Jude -
	IV	Weiß -	Hist. -	Hist. -	Hist. -	Weiß -	Weiß -	Weiß -	Weiß -
	V	Hist. +	Weiß +	Hist. +	Hist. +	Weiß -	Teno- sin +	Teno- sin +	Teno- sin +
	VI	Weiß -	Weiß -	Weiß -	Weiß -	Weiß -	Weiß -	Weiß -	Weiß -
	VII	Hist. +	Hist. +	Hist. +	Hist. +	Hist. +	Hist. +	Hist. +	Hist. +

Erläuterung bezügl. der Impfungsgruppen:

Gruppe I: Gesamtmenge = 0,6 ccm Serum in 3 Dosen à 2 ccm Verdünnung.

Gruppe II: Gesamtmenge = 4,4 ccm Serum in 14 Dosen à 2 ccm Verdünnung.

Weitere Erläuterungen siehe Text.

wie ja auch nicht anders zu erwarten war, denn das Unterart-  
Proten „Mensch“ und eventuelle dem jüdischen Blutserum eigene  
Protene, die auch im „weißen“ Serum vorhanden waren, traten in  
Reaktion und wurden abgesättigt. Nachdem der Muskel sich wieder  
gestreckt (dieses kann in verschieden langer Zeit erfolgen) und  
eine kurze Strecke gezeichnet hatte, wurde bei II dasselbe hinzuge-  
geben. Der Muskel reagierte nicht mehr darauf, es waren also alle

für jüdisches Serum spezifischen  
Antikörper abgesättigt. Der nun  
folgende Pfeil besagt nur eine  
Durchspülung des Bades mit vor-  
gewärmter frischer Tyrode-Lö-  
sung, um die von der letzten  
Zugabe noch darinbefindlichen  
Antigenmengen restlos zu ent-  
fernen. Hierauf wurde bei III  
„weißes“ Serum hinzugegeben.  
War nun ein Unterschied in  
qualitativer Hinsicht vorhanden,  
dann mußte, da ja anzunehmen

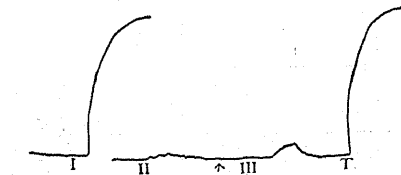


Abb. 1. Tier Nr. 7/39, System: Anti-  
weiß, Injektionsgruppe II (siehe Tabelle  
S. 955). Inkubation: 70 Tage. Uterus-  
horn a.: Reaktionen mit je 2 ccm  
Serum-Verdünnung 0,2 : 10 : I Jude, II  
dasselbe, III Weiß, T = Tenosin.

Die Pfeile (†) bedeuten Spülung mit Tyrode-  
Lösung.

war, daß „Weiß“ als Homologes noch eigene artspezifische Protene  
enthielt, jetzt wiederum eine Reaktion erfolgen, welche auf der  
Absättigung die-  
ser für „Weiß“  
spezifischen Pro-  
tene beruht hätte.  
Diese Reaktion er-  
folgte, wie er-  
sichtlich, nicht  
(die kleine Er-  
höhung ist keine  
Reaktion, nicht

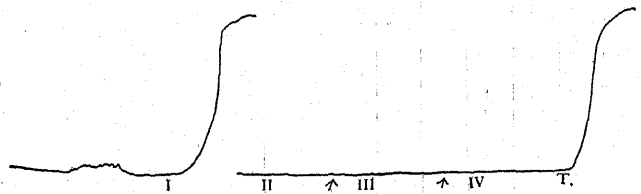


Abb. 2. Tier Nr. 7/39 Uterushorn b.: Dieselben Daten wie in  
Abb. 1.: Reaktionen: I Gelb, II Dasselbe, III Jude, IV Weiß,  
T = Tenosin.

einmal der Beginn einer solchen, da derartige Bewegungen des Muskels  
häufiger vorkommen). Erst bei der Zugabe von Tenosin bei T erfolgte  
eine erneute Kontraktion des Hornes.

Zum Beweis, daß negative Endreaktionen nicht auf Erschlaffungs-,  
Ermüdungs- oder Todeserscheinungen beruhen, setzt man als Schluß-  
reaktion eines jeden Verlaufes einige Tropfen Histaminlösung oder  
Tenosin, also unspezifische Muskelgifte, hinzu, deren positiver oder  
negativer Ausfall Leben oder Tod des Muskels anzeigt. Gewöhnlich  
lebt der Muskel in dem Bade etliche Stunden.

Ganz ähnlich ist die Reaktionsfolge des Parallelhornes und des  
Tieres Nr. 41. (Abb. 3 u. 4.) Bei dem Tiere Nr. 41 ist zwischen die

erste Reaktion und die Zugabe von „weiß“ noch eine weitere Zugabe eingeschaltet worden, welche an beiden Muskeln, ebenso, wie die Reaktion „Weiß“ negativ verliefen. (vergl. Abb. 2).

Betrachten wir nun einmal die Tabelle 2, so müssen wir feststellen, daß bei allen zur Reaktion geführten Tieren nach der ersten Reaktion, die bei allen Tieren positiv ausfiel, keine weitere positive Reaktion auftrat, nur eben bei dem Zusatz von Histamin und Tenosin (s.o.). Es

wurden also schon bei der ersten Reaktion sämtliche gegen „Weiß“ gerichteten Antikörper abgesättigt. Wäre ein qualitativer Unterschied zwischen „Weiß“ und den anderen Seren vorhanden gewesen, dann hätte die Reaktion mit „Weiß“ als dem Homologen positiv ausfallen

müssen. Waren dagegen schon bei der ersten Zugabe alle für „Weiß“ spezifischen Antikörper abgesättigt, so war es naturgemäß auch nicht möglich, daß bei einem weiteren Zusatz eines anderen Serums (Gelb, Schwarz etc.) noch eine positive Reaktion erfolgen konnte.

Nach diesen Ergebnissen muß man wohl ein Vorhandensein serologisch faßbarer, qualitativer Unterschiede der Rassen-Seren im Sinne BRUCK's ablehnen.

### Versuch der theoretischen Auswertung

Wie aus dem vorangehenden experimentellen Teil hervorgeht, ergeben unsere Untersuchungen ein völlig anderes Bild als die Arbeit BRUCK's. An der experimentellen Richtigkeit der BRUCK'schen Untersuchung zu zweifeln, haben wir keinen Grund. Es besteht also nur die Aufgabe der Aufklärung dieser Diskrepanz. Ein ähnlicher Mangel an Übereinstimmung besteht bekanntlich bezüglich der „großen Systematik“ zwischen den Ergebnissen der Präzipitationsuntersuchungen MEZ' und den Untersuchungen mit der Anaphylaxiemethode. Man kann hier von einem „Reichweitenparadoxon“ sprechen. Denn die empfindlichere Anaphylaxiemethode, mit deren

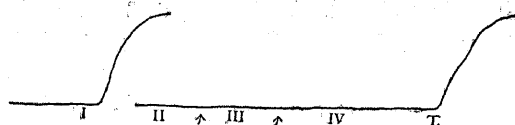


Abb. 3. Tier Nr. 9/41, System: Anti-Weiß, Injektionsgruppe I (siehe Tabelle S. 955) Inkubation: 123 Tage. Uterushorn a.: Reaktionen mit je 2 cem Serum-Verdünnung 0,2:10: I Schwarz, II Dasselbe, III Jude, IV Weiß, T = Tenosin.

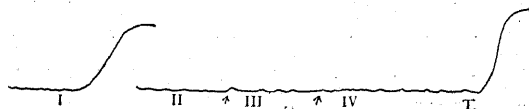


Abb. 4. Tier Nr. 9/41, Uterushorn b. Dieselben Daten wie in Abb. 3. Reaktionen: I Jude, II Dasselbe, III Gelb, IV Weiß, T = Tenosin.

Hilfe man wesentlich kleinere Eiweißmengen als mit der Präzipitationsmethode nachweisen kann, gestattet im allgemeinen keine Reaktionen zwischen systematisch verwandten Familien, während die weniger empfindlichere Methode der Präzipitation nach MEZ und anderen mit Leichtigkeit Eiweißgemeinschaft zwischen den Familien zu entdecken gestattet. Zum Teil kann dieses Paradoxon durch den Hinweis aufgeklärt werden, daß die Präzipitation nicht nur Eiweißkörper, sondern auch Lipide erfasst (MORITZ: 1929).

Im Prinzip sind die an die Methoden zu stellenden Anforderungen nun die gleichen, einerlei, ob wir Eiweißdifferenzen bei nahe verwandten oder Eiweißgemeinschaft bei entfernt verwandten systematischen Einheiten aufklären wollen. In beiden Fällen handelt es sich darum, eine sehr geringe Menge der für den Ausfall der Reaktion entscheidenden Eiweißkörper neben großen Mengen anderer Eiweißkörper zur Antikörperbildung zu bringen. Mit anderen Worten: Es sind Maßnahmen zu treffen, welche verhindern, daß die in geringer Menge vorhandenen entscheidenden Partial—Antigene (Partial-Rezeptoren, Protene, Proteale (MOLLISON 1933) ) infolge der Konkurrenz der Antigene nach der Injektion garnicht zur Antikörperbildung Anlaß geben. Bei den Versuchen zur Feststellung serologischer Gemeinsamkeiten zwischen entfernter verwandten Einheiten hat man von jeher dazu den Weg beschritten, möglichst hochwertige Seren durch wiederholte Injektion zu erzielen. Auch bei den eigenen Untersuchungen zur Bastardserologie (bei denen es also auf Erfassung geringer Mengen unterscheidenden Eiweißes ankam) erwiesen sich im allgemeinen wiederholte Injektionen und langausgedehnte Inkubationszeiten als günstig.

Es muß dementsprechend befremden, daß BRUCK ausdrücklich nur wenig hochwertige Antiseren (solche von geringem Titer) anwendet und erklärt, daß nur mit solchen seine Ergebnisse erhalten werden konnten.

Die Aufklärung der Unterschiede in den Ergebnissen dürfte aber durch grundsätzliche Erwägungen möglich sein. Bei einer früheren Gelegenheit wurde darauf hingewiesen, daß es unmöglich sei, aus den Ergebnissen der titrierenden serologischen Methoden (Präzipitation, Komplementbindung, Agglutination, kurz alle, bei denen man mit dem Begriff des „Titers“ arbeitet) eindeutige Schlüsse über das Ausmaß der Eiweißgemeinschaft mehrerer systematischer Einheiten mit einer anderen (die als „homologes Antigen“ zur Herstellung der Antiserums diene) zu ziehen (MORITZ: 1934a).

Die Gründe dafür sind: 1. Das Fehlen einer rationellen Bezugsgröße. Denn in die Reaktion geht notwendig der Konzentrationsbegriff ein, in diesen das Molekulargewicht. Dieses ist uns für die



reagierenden Stoffe nicht bekannt. 2. Es fehlt im allgemeinen bei diesen Untersuchungen der Nachweis, daß die Reaktion, deren Stärke (Präzipitations-, Komplementbindungs-, Agglutinationstiter) beurteilt wird, auf der qualitativ gleichen Grundlage beruht. Mit anderen Worten, wenn mit dem System Anti—A das Antigen B bis 1:10000, das Antigen C bis 1:5000 reagiert, so können diese Reaktionen auf qualitativ ganz verschiedener Grundlage beruhen, also auch quantitativ unvergleichbar sein.

Es wurde daher vorgeschlagen, alle diese titrierenden serologischen Reaktionen im Bereich der Verwandtschaftsforschung als „Pseudoquantitative“ Reaktionen zu bezeichnen (MORITZ: 1934a).

Die einzigen Aussagen, welche diese Reaktionen ermöglichen, sind: 1. Zwei systematische Einheiten haben Eiweißgemeinschaft, oder 2. sie haben keine Eiweißgemeinschaft.

Gänzlich unmöglich ist es aber natürlich, auf Grund der Ergebnisse pseudoquantitativer Methoden ins Einzelne gehende qualitative Aussagen zu machen. Wir gelangen dann zu pseudoqualitativen Ergebnissen, wie das bereits früher (MORITZ: 1934a) für die MOLLISONschen (1933) Untersuchungen an Affen nachgewiesen wurde. Allerdings wurde damals der Ausdruck „pseudoqualitativ“ noch nicht verwendet. Doch wurde darauf hingewiesen, daß der Begriff der „Proteale“, der formell unserm „Proten“-Begriff gleicht, in unzulässiger Weise von MOLLISON verwendet wurde. Das Gleiche gilt auch von den BRUCKSchen Ergebnissen. Da sie mit Hilfe einer titrierenden Methode gewonnen wurden, lassen sie keine eindeutigen qualitativen Schlüsse zu.

Solche eindeutigen qualitativen Schlüsse, die zur Zeit das Maximum des Erreichbaren darstellen, können auf serologischem Gebiete nur durch Anwendung von Absättigungsreaktionen erhalten werden, wie sie weiter oben, im experimentellen Teil im Grundsatz gekennzeichnet wurden.

Es entsteht noch zum Schluß nun die Frage, wie dann aber die BRUCKSchen Ergebnisse zustande gekommen sein können. Nach dem Ausfall unserer Absättigungsreaktionen und im Anbetracht dessen, daß die BRUCKSchen Ergebnisse mit Seren niedrigen Titers erhalten wurden, ist es unwahrscheinlich, daß ihnen wirklich qualitative Unterschiede zu Grunde liegen. Nehmen wir aber an, daß sich die untersuchten Seren qualitativ gleich zusammen setzen aus den gleichen Partialantigenen (Protenen, Partialrezeptoren, Protealen), dann könnte möglicherweise eine Differenzierung vorgetäuscht werden, wenn die relativen Mengenverhältnisse der einzelnen Partialantigene bei den Seren der untersuchten Rassen mengenmäßig variieren. Hier eine Entscheidung zu fällen ist derzeit ganz unmöglich. Es sei jedoch

darauf hingewiesen, daß bei Verwendung von Seren geringer Titer unter Voraussetzung der Konkurrenzerscheinung folgender Fall eintreten kann: Rasse A besitze lediglich die Protene a und b in den gleichen Mengen. Rasse B besitze viel a, daneben aber von den Proteinen b, c, d je so wenig, daß diese bei geringem Immunitätstiter nicht durch Antikörper im Antiserum vertreten sind. Dann wird die Rasse B, obgleich sie an serologischen Merkmalen reicher ist, im Versuch als die ärmere erscheinen können.

Fassen wir zusammen, so erkennen wir, 1. daß bis jetzt eine eindeutige serologische qualitative Unterscheidung von biologischen Rassen nicht möglich war, wie am Beispiel von Menschenrassen gezeigt wurde.

2. daß die Auswertung serologischer Methoden in der Verwandtschaftsforschung eine sehr genaue Methodenkritik erfordert, wie am Beispiel der pseudoquantitativen und pseudoqualitativen Methode und Aussage erläutert wurde.

#### Literaturverzeichnis

- Bruck, C. Die biologische Differenzierung von Affenarten und menschlichen Rassen durch spezifische Blutreaktion. Berliner Klin. Wochenschr. 26: 793, 1907.
- Dale, H. H. The anaphylactic reaction of plain muscle in the guinea-pig. J. of Pharmacol. 4, 1912.
- Magnus, u. Friendenthal, Über die Spezifität der Verwandtschaftsreaktion der Pflanze. Ber. dtsch. botan. Ges. 25, 1907.
- Magnus, W. Weitere Ergebnisse der Serodiagnostik für die theoretische und angewandte Botanik. Ber. dtsch. botan. Ges. 26, 1908.
- Mollison, Th. Phylogenie des Menschen. Handb. d. Vererbungswiss. 18, 1933.
- Moritz, O. Zur Kritik der Phytoserologie. Biol. Zentralbl. 48, 1928.
- Weitere Beiträge zur Kritik und zum Ausbau phytoserologischer Methodik. Planta 7, 5, 1929.
  - Prinzipien und Beispiele der Anwendung phytoserologischer Methodik. Planta 15, 1932.
  - Serologische Untersuchungen an Getreidebastarden. Ber. dtsch. botan. Ges. 51, 1933.
  - Die botanische Serologie. Cohn's Beitr. z. Biolog. d. Pflanzen. 22: 51, 1934.
  - Über serologische Verwandtschaftsforschung. Der Züchter 10, 1934.
  - u. vom Berg, H. Serologische Studien über das Linswickenproblem. Biol. Zentralbl. 51: 6, 1931.
- Nuttall, Blood immunity and blood relationship. Cambridge 1904.
- Uhlenhuth, P. Ein Verfahren zur biologischen Untersuchung von Blut verwandter Tiere. Dtsch. Mediz. Wochenschr. 1905.
- Wells, H. G. Die chemischen Anschauungen über Immunitätsvorgänge. Jena, Gustav Fischer 1927.

## Significance of Cytogenetic Alterations Induced by High Frequency Radiation in *Nicotiana* Species \*

By

**T. H. Goodspeed**

University of California, Berkeley

The effectiveness of x-radiation in inducing gene mutation and chromosome reorganization in living organisms, has been adequately demonstrated by the work of many investigators. Studies under way during the past nine years in the University of California Botanical Garden have analyzed many products and byproducts of high frequency radiation applied to sex cells, seeds and growing points of species of the genus *Nicotiana*. The effectiveness of such treatment in the case of these species lies not only in its capacity to produce mutations in genes or structural alterations in chromosomes, but also in its ability through a single original alteration to initiate a succession of changes continued over a considerable series of generations, the products of which are not necessarily related directly to the original radiation effect. Such a range of induced variation is, in our experience, to be referred to an initial alteration of chromosome structure or organization. In nature similar clusters of variations have arisen and doubtless in many cases they are to be assigned to chromosome alterations naturally induced and similar to those produced in *Nicotiana* species by high frequency radiation. In this connection attention might be called to the obvious fact that evolution has not proceeded at a uniform rate in all organisms or even among closely related ones, and the fact that certain species or groups of species tend to vary more than others may indicate a certain parallelism between the character of induced and natural variation.

Three cytogenetic states, among others, which have been a product of x-radiation of *Nicotiana* species, have been most thoroughly analyzed: (1) continued segregation following induced fragmentation and translocation, (2) variation caused by repeated disruption of a complex chromosome mechanism initially induced by x-radiation, (3) the asynaptic condition arising as a result of a recessive gene mutation similarly induced. This report contains a brief résumé of the evidence concerning these three cytogenetic states, together with comment upon their combined significance.

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### First cytogenetic state

In progenies derived from a single x-rayed sex cell of *Nicotiana Tabacum* it has been possible over a period of years to produce a multiplicity of distinct morphological types because subtractions from, as well as additions to, the genom of this species may be viable in the homozygous condition (1). In tetraploid races or amphidiploids involving closely related species recessive alterations due to loss may not produce distinct morphological variation because of the presence of all or a majority of the genes in duplicate. On the other hand, when the amphidiploid species, like *N. Tabacum*, has arisen from less closely related species which differ from each other widely in genotype, many such losses should be expressed in detectable recessive effects. These recessive types may survive because of the presence of similar genes essential for viability in the unaltered parental set present in the amphidiploid. Thus, a series of homozygous losses has been established from a few translocations and fragmentations and has given rise to morphologically distinct recessive types. They represent individual gradations in extent of distinction in external morphology when compared to control and, indeed, it may be said that there is a general parallelism between extent of visible chromosome alteration shown by an x-ray *N. Tabacum* derivative and the extent of its distinction from control in external morphology. For example, one of the eight pure-breeding types obtained from x-radiation of a single sex cell of *N. Tabacum* differs from control in only a few characters, and shows no visible alterations in genom, whereas another is strikingly different in many characters and its genom gives evidence of having undergone complex chromosome reorganization.

Certain, if not many, plant species of economic importance, like *N. Tabacum*, have been shown to be of amphidiploid origin. Their capacity to exhibit variation and thus undergo successful selection is to be assigned in considerable part to a viability which is retained through the presence of one set of genes after quantitative or qualitative alteration of their original duplicates. Thus, the demonstrated lability of the gene system of *N. Tabacum* after treatment with high frequency radiation is, as already noted, referable to the presence of the two complete chromosome sets of each parent. In this connection it should be noted that Demerec (2) has pointed out the importance of the addition of loci in the origin of a new species. The duplication of a few loci must be followed by their differentiation before a new race is established. This process of differentiation may be quite time consuming, but once attained the production of additional forms is possible through losses of genes. These points are illustrated by the products of the cytogenetic state under consideration. Thus, the

eight pure breeding x-ray derivatives in *N. Tabacum* were rapidly established through loss of genes which were duplicated in the amphidiploid origin of this species. On the other hand, certain of the other derivative types not as yet obtained in pure breeding condition owe their distinction from control in external morphology to duplicate genes present in chromosome fragments which are not essential for viability and may therefore, in succeeding generations, be present in different number to give a range in the morphological expression of the type.

### Second cytogenetic state

This case involves the presence of an unstable chromosome mechanism induced by x-radiation and will be given only brief consideration at this point. In this second cytogenetic state we have evidence that the differentiation of the two parental gene sets of *N. Tabacum* has resulted in the establishment, in the F chromosome at least, of certain genes essential for viability. Thus in *deformed*, a derivative distinguished by a greater or lesser degree of tissue abnormality, the physical attachment of the F chromosomes induced by x-radiation may result in their mitotic and meiotic elimination with consequent lethality during the course of tissue and gamete formation. The presence of the attachment also gives rise to a complexity in chromosome behavior during meiosis. The disruption, particularly at IIA, of the chromosome bridges which form as a consequence of the attachment results in the production of a series of F chromosome remnants whose survival depends upon the retention of an insertion region. The viability of a zygote from gametes produced following this disruption depends upon its receiving from a fragment or some other F chromosome a certain viability region characteristic of this chromosome. As a result of the operation of this complex chromosome mechanism both plus and minus chromosomal types may appear (*fluted*, *long flowers* and variants of these two types), and also pure breeding types (*coral* and *mammoth*) together with types showing tissue abnormalities (*deformed*) or color variegation (*carmine-coral*) produced by mitotic irregularities in distribution of the F chromosomes or their fragments. In other words, despite the viability limitation a wide range of products and byproducts of the initially x-ray induced chromosome alteration have been secured.

### Third cytogenetic state

In this case we are dealing with a product of x-radiation of *N. sylvestris*, a 12-paired species, a progenitor of which entered into the amphidiploid origin of 24-paired *N. Tabacum*. Among a number of gene mutations induced by x-rays in this species a recessive gene

effect which appeared in  $X_2$  and which causes asynapsis of homologous chromosomes will be briefly described.

Seven generations have been grown from the original asynaptic parent, and in them an extended series of hyperploid plants has appeared. Thus, 11 primary trisomic types have been identified, together with a number of simple trisomics which appear to be similar to the secondary and tertiary types described by Blakeslee in *Datura*, over 30 double trisomics, more than 10 tetrasomics and at least 6 triple trisomics. The trisomic types differ from the diploid in height and habit of plant; in shape, size and disposition of leaves; in flower length, breadth and shape and in the length and relative position of stamens and style. In other words, the genic balance of each chromosome is different from that of the complement as a whole and affects a large number of plant characters. It is also clear that the expression of any one character considered "normal" for the species *N. sylvestris* is conditioned by the ultimate balance between the various chromosomal unbalances of each of the 12 chromosomes in its haploid set. For example, the trisomic types called *enlarged* and *recurved* increase the length of the flower, whereas *stubby*, *puckered* and *compact* decrease flower length, and similar variations are characteristic of trisomic types where leaf shape and leaf color are concerned.

As in certain other described cases of asynapsis, its extent varies with changes in environmental conditions and particularly temperature. Thus, during hot weather asynapsis is increased, an effect also characteristic of the  $2n + 1$  and  $2n + 2$  derivatives when compared with the extent of asynapsis in  $2n$  plants.

Through backcrossing to the non-asynaptic diploid, trisomic lines devoid of asynapsis have been produced. The distinction between the proportion of variants in such lines and that from asynaptic plants is striking; for while the former usually contain only  $2n + 1$  and  $2n$  plants, the latter have given as many as 16 different chromosomal types in a population of 68 plants, only 44% of the population being unchanged in chromosome constitution. Although, as already stated, asynapsis is expressed more completely in hyperploid plants than in diploids, the asynaptic condition is transmitted as effectively through one as through the other so that the source of variation is lost only after crossing with plants carrying the dominant non-asynaptic gene.

In addition to the asynaptic derivatives above mentioned other chromosomal variants have been obtained—a haploid and several plants with fragment rather than whole chromosomes added to the  $2n$  complement. The fact that chromosomes become fragmented and translocated in lines derived from asynaptic plants makes possible

the establishment of additional chromosomal types. In other words, in this third cytogenetic state, as in the other two, an initial alteration in the genetic material induced by x-rays produces, secondarily, an extended series of chromosomal variants.

### Discussion

The problem of the origin, nature and significance of gene and of chromosome alterations was debated long before high frequency radiation was shown effective in producing them, and the results of recent genetic investigation appear to substantiate certain of the earlier contentions such as that of Bateson, who looked upon all recessive gene mutations as losses. The shift of emphasis in modern genetics toward analysis of quantitative chromosomal variations rather than of the results of transgenation can largely be ascribed to the capacity of high frequency radiations to furnish ample material for the study of chromosome alterations. In addition, the identification and study of induced quantitative chromosomal variations has led to a broader recognition of their occurrence under natural conditions. Indeed, the variation which in origin can be referred to structural chromosome alteration is beginning to be looked upon as a significant evolutionary process. For it is clear that differences in genic organization between closely related but distinct species could be accounted for by the accumulation of successive structural genome alterations no one of which would be lethal. While transgenations (involving molecular rearrangement of genes) may occur concurrently with, and independently of, quantitative chromosome reorganization, it must be remembered that the influence of losses due to the latter effect might give results indistinguishable from those assigned to transgenations, particularly when lethality does not follow the initial change because of the presence of the two parental sets of genes.

It is evident that the accumulation of recessive gene mutations accompanies and is to a considerable extent responsible for species differentiation. On the assumption that such mutations represent losses, one encounters the difficulty, appreciated by the contestants in the "presence-absence" controversy, that evolution cannot continue solely through accumulation of losses. On the other hand, if such losses follow duplication of groups of genes, they will then serve to differentiate such duplications.

That this sequence of events can occur is shown by the cytogenetics of derivative races obtained from x-radiation of *Nicotiana Tabacum*. Thus, as has been said, the amphidiploid origin of this species provides a large number of duplications permitting the establishment of many homozygous losses. By contrast *N. sylvestris*, one



of the two species whose progenitors entered into the origin of *N. Tabacum*, has given only one x-ray derivative whose origin is clearly via loss, and this plant was small and weak. On the plus side, however, a large number of chromosomal variants have been obtained.

The three cytogenetic states to which this report has been limited have, in common, the capacity of continuous production of new chromosomal types. The mechanism involved in each case is different but in all three the alteration, initially induced by high frequency radiation, influences chromosome behavior, a most potent source of further quantitative chromosomal variation and one which may produce unlimited material upon which evolutionary processes can operate. The extent to which structural chromosome alterations in *Oenothera* have entered into species differentiation and the fact that in *Datura stramonium* the differences in genic organization between the various derivative races have had their origin in structural changes of the chromosomes illustrates the evolutionary significance of such structural alterations and indicates that they occur under natural conditions. Whether or not such alterations represent a major evolutionary mechanism is, perhaps, a question, but at least it is probable that the degree of differentiation between two species is proportional to the number of structural distinctions between their genomes. A case in point is the cytogenetic situation in the group of 12-paired *Nicotiana* species to which *paniculata*, *solanifolia* and *Raimondii* belong. All hybrids which have been obtained between these species show a large amount of conjugation indicating a high degree of similarity in genic content and organization. Similarly these three species are strikingly similar in somatic chromosome morphology. On the other hand, in the hybrids within this group there is evidence at IM of structural differences between the chromosomes of any two complements. Thus, in  $F_1$  *Raimondii*  $\times$  *solanifolia* at least 6 of the 12 bivalents present are either heteromorphic or unequally aligned, indicating that translocations and inversions have taken place during the differentiation of the parental species involved. If, as in this illustration, quantitative chromosomal variation is of significance in the evolution of species then it is clear that mechanisms, such as those described in this report, which are capable of continually producing such variation are not solely novelties but are important initiators of species differentiation.

#### Literature

- 1) Goodspeed, T. H. and P. Avery: The cytogenetics of fourteen types derived from a single x-rayed sex cell of *Nicotiana Tabacum*.—*Jour. of Genetics*, 29: 327-353. October 1934.
- 2) Demerec, M.: Role of genes in evolution. *Amer. Nat.* 69: 125-138. Mar.-Apr. 1935.

## On the Autopolyploids of the Rape<sup>1)</sup>

By

T. Morinaga and H. Kuriyama

Plant-Breeding Laboratory, Kyushu Imperial University

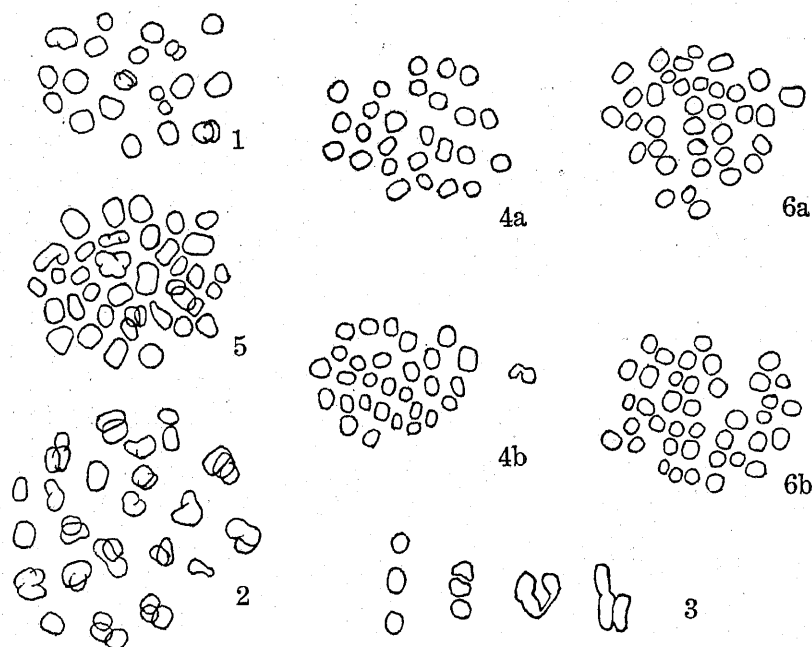
In the spring of 1935, Mr. K. Arashi, in charge of the rape breeding in the Experimental Station at Fukuoka, found a number of highly sterile plants in an otherwise promising strain selected from 'Wase-Chôsen', a common variety of the rape (*Brassica Napella* Chaix). According to one of the writers' (K.) investigations, those spontaneous plants were diploid in all external respects, though the styles and filaments of their flowers were more thickset, their leaves more glaucous, and their branches grew more nearly upright than those of the normal plant. About 2/3 of the pollen-grains of such plants were abnormal and appeared to be functionless. The anthers of more than 20 such sterile plants and some normal ones were fixed separately with Bouin's solution, and permanent preparations for the study of microsporogenesis were made by the paraffin method. The normal plant showed 19 bivalent chromosomes (Fig. 1) in the heterotypic metaphase, and the division processes were carried out regularly. The sterile plants, though possessed of almost the diploid number of chromosomes, so far as the writers noted, revealed, in the heterotypic metaphase, several univalents for some reason or other. The univalents usually lag behind in the anaphase, and the formation of restitution nuclei was not infrequently met with. Dyad formations instead of tetrads occurred also in an abnormally high frequency in these plants.

In the autumn of 1935, the writers sowed the seeds produced by 7 such highly sterile plants, obtaining 7 lines, T<sub>26</sub>, T<sub>27</sub>, T<sub>28</sub>...T<sub>32</sub>, which in all included 79 individuals. Four autopolyploid plants were discovered among those plants.

*Autotriploid*: Three individuals, namely T<sub>27</sub>-702, T<sub>28</sub>-715 and T<sub>28</sub>-719, though they were somewhat dwarfed, set on rather thick leaves and stalks, and had slightly larger flowers than the normal plant. The anthers burst easily and the pollen-grains, though not uniform in size, were stained well with aceto-carmin. The microsporogenesis of those 3 plants was studied by both the aceto-carmin smear and the ordinary paraffin methods. The following descriptions,

1) Contributions from the Institute of Agronomy, Kyushu Imperial University, No. 57.

however, were based chiefly upon the results of the smear method. The heterotypic chromosome numbers of the plants were not constant, and the writers counted 21–24 chromosomes, which, except the univalents, arranged themselves well on the equatorial plane. The shape of the majority of the chromosomes was complicated, and the rest showed either the normal shape of a bivalent or the shape of a univalent (Fig. 2). Those chromosomes of a complicated shape were easily seen to be trivalents in their side views (Fig. 3). The trivalent



Figs. 1-6. 1. A polar view of the heterotypic metaphase of the normal diploid.  $\times 2250$ . 2. A polar view of the heterotypic metaphase of the triploid plant.  $\times 2550$ . 3. Side views of trivalent chromosomes.  $\times 2550$ . 4a, b. Polar views of the sister homotypic plates of the triploid plant.  $\times 2250$ . 5. A polar view of the heterotypic metaphase of the tetraploid plant.  $\times 2250$ . 6a, b. polar views of the sister homotypic plates of the tetraploid plant.  $\times 2250$ .

often seems to disjoin early, producing a bivalent and a univalent situated close together. In the anaphase which followed, 1–6 lagging chromosomes were usually observed. The lagging chromosome sooner or later divided into two, and the halves moved to each pole, though they failed sometimes to be included in the daughter nuclei. In the homotypic metaphase there were found 26–30 chromosomes, the average number being 28.3 (Fig. 4). A small number of chromosomes were also situated out of the equatorial plane. The double of the homotypic chromosome number, or the total number of chromosomes

in the two homotypic nuclei in a microsporocyte, thus agrees closely with the triploid number (57) of the species. Though in the homotypic anaphase a few lagging chromosomes were met with, the division proceeded normally as regards other points. From the observations above mentioned the writers concluded that the individuals,  $T_{27}$ -702,  $T_{28}$ -715 and  $T_{28}$ -719 were autotriploids produced by the union of a normal and a diploid gamete, the latter caused from the restitution nucleus formation.

*Autotetraploid:* The individual  $T_{27}$ -706 was a plant of a dwarf gigas type with thick leaves and stems, and flowers slightly larger than the normal. The pollen-grains were not equal in size, but scarcely any empty grains were observed. The smallest number of chromosomes counted in the heterotypic metaphase was 23, and the greatest number exceeded 30 (Fig. 5). In the smear preparation some chromosomes were presumed by their size and shape to be tetravalent, but it was impossible to determine the valency for all of the chromosomes. In the heterotypic anaphase a few, in one case as many as 5, lagging chromosomes were observed. The average number of the homotypic chromosomes, though counted only for 4 metaphasic plates, was 37.8. Two sister equatorial plates in the microsporocyte depicted in Fig. 6 contain respectively 35 and 39 chromosomes. From these observations (which are not regarded as satisfactory) the plant was assumed to be an autotetraploid, originated by the union of two diploid gametes formed by the restitution process.

Various kinds of numbers showing the grade of fertility of the autotriploid and autotetraploid are shown in the following table.

Table showing the fertility of the auto-polyploids of the rape

	Length of silique	Number of placentas per silique	Number of perfect seeds per silique	Number of imperfect seeds per silique	Number of perfect seeds	Total number of seeds
					Number of placentas	Number of placentas
Diploid	4.2 cm	21.9	13.5	1.5	61.6%	68.5%
Triploid ( $T_{28}$ -715)	2.6	18.5	3.9	0.5	21.1	23.8
Tetraploid ( $T_{27}$ -706)	2.3	14.5	0.5	1.5	3.4	13.8

Many thanks are due to Mr. K. Arashi for the seeds which he has kindly supplied the writers with as material.

### Zytologische Untersuchungen der Bryophyten, III. Die Morphologie des Spermatozoids von *Reboulia hemisphaerica*<sup>1)</sup>

Von

**Tadamasa Miduno**  
Kaiserliche Universität Tokyo

Als Fortsetzung meiner vorhergehenden Untersuchungen habe ich weiter Forschungen über die Morphologie der Spermatozoiden von *Reboulia hemisphaerica* (L.) Raddi. gemacht. Da die Spermatozoiden desselben einige besondere Eigenschaften haben, möchte ich hier die Einzelheiten veröffentlichen.

#### Methode und Material

Das in dieser Forschung gebrauchte Material wurde in Zinmuzi (Kanagawa-Prefektur) gesammelt. Gewöhnlich findet man den Thallus auf ziemlich getrocknetem Boden oder Gestein. Die kreisförmigen Antheridienstände entstehen auf dem Thallus und haben keinen Stengel wie bei *Conocephalus*, die Größe der Antheridienstände aber ist kleiner als die von *Conocephalus*. Die Reifezeit des Antheridiums von *Reboulia hemisphaerica* ist Oktober. Ein Teil des Thallus wurde vom Boden genommen und etwas davon im Laboratorium sofort, und der Reste nach einigen Tagen untersucht. In beiden Fällen wurden Spermatozoiden gewonnen. Wenn Man das Antheridium aus dem Thallus abschneidet, es umgekehrt auf den Objektträger stellt und darauf ein Tröpfchen Leitungswasser hinzufügt, so kommen nach 5–10 Minuten sehr viele Spermatozoiden heraus. Nach Entfernung des Antheridiums, welches schon die Spermatozoiden ejakuliert hatte, wurde schwimmende Spermatozoiden enthaltendes Wasser auf der Flamme, im Thermostat, oder an der Luft ausgetrocknet. Gewöhnlich, vor dem Austrocknen des Wassers, wurde der Dampf der Osmiumsäure zur Fixierung gebraucht. In dem vorliegenden Experiment aber zeigten die Form und der Färbungszustand des Spermatozoids in beiden Fällen keinen Unterschied. Folglich, wenn man die Osmiumsäure nicht erhalten kann, genügt allein das Austrocknen für die Färbung und Beobachtung des Spermatozoids. Für die Färbung wurde meistens Gentiana-violett gebraucht, auch Carbofuchsin zugleich verwendet. Bei der Färbung fügte ich auf den Punkt, an welchem die Spermatozoiden getrocknet wurden, ein oder zwei Tröpfchen Gentianaviolettlösung hinzu. Nach 1–3 Minuten wurde das Färbungsmittel mit Wasser

1) Contributions from the Divisions of Plant-Morphology and of Genetics, Botanical Institute, Faculty of Science, Tokyo Imperial University, No. 178.

ausgewaschen wobei zugleich Differenzierung erzielt wurde. Man muß das Wasser, welches für das Auswaschen gebraucht wurde, in dem Thermostaten ziemlich schnell austrocknen, um die Differenzierung nicht weiter fortschreiten zu lassen. Zuletzt wurde das Material mit Balsam eingeschlossen und unter ein Deckglas gelegt. Alle Mikrophotographien wurden mit Hilfe von Leitz "Makam" aufgenommen und alle Zeichnungen wurden mit dem Zeiss-Zeichenapparat nach Abbe gemacht. Zu der Messung des Spermatozoids brauchte ich die Methode, die in den früheren Untersuchungen (Miduno 1934, 1935) zur Verwendung kam.

### Die Morphologie des Spermatozoids

In der vorliegenden Abhandlung ist die Bezeichnung eines jeden Teils des Spermatozoids wie in der vorhergehenden Arbeit (Miduno 1934) vorgenommen. Auch das Spermatozoid der betreffenden Art kann man in vier Teile, nämlich in das Kernstück, die Zilie, die Stammsubstanz und das Plasmastück, einteilen.

Das Kernstück: Die Form des Kernstückes ist ähnlich der von *Wiesnerella* und *Dumortiera*; nach Fixierung und Färbung aber ist das Kernstück dieser Art stärker gekrümmt als das der oben genannten zwei Arten. Die Dicke des Kernstückes beträgt am mittleren Teil etwa  $0.65 \mu$ , also auffallend dünner als diejenigen von anderen Arten, ausgenommen nur das von *Marchantia*, welches das dünnste unter den bisher vom Verfasser beobachteten darstellt. Im lebenden Zustand zeigt es eine  $1\frac{1}{2}$  malige Windung, im fixierten Zustand aber weist es keine Spiralwindung mehr auf, sondern streckt sich meistens nach der Hufeisenform oder der Sichelform (Abb. 1). Wo unreife Spermatozoiden ejakuliert wurden, zeigen die Kernstücke runde Form, und entlang der Außenseite des Kernstückes laufen die Zilien in gleicher Richtung mit dem Kernstück. Das Kernstück wird mit Genticianaviolett oder Carbofuchsin einheitlich dunkel gefärbt, und beide Enden desselben sind zugespitzt. Die Länge des Kernstückes beträgt nach dem Mittelwert von 200 Individuen  $10.38 \mu$  (Abb. 2), somit ist das Spermatozoid von *Reboulia hemisphaerica* am kleinsten unter den Spermatozoiden der Lebermoose, welche der Verfasser bis jetzt beobachtet hat. Als mißgebildete Form wurden einige Spermatozoiden mit ungewöhnlich kleinem Kernstück ( $6.9 \mu$ ) beobachtet (Abb. 11). Es ist denkbar,

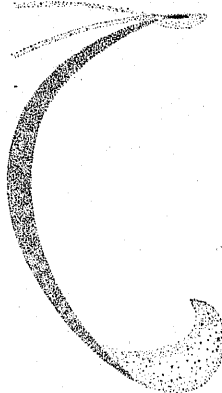


Abb. 1. Das normale Spermatozoid. Die Zilien sind nur an ihrem proximalen Teil gezeichnet. ca. 6700 $\times$ .

daß diese Zwergspermatozoiden noch unreif sind; die Zilien von

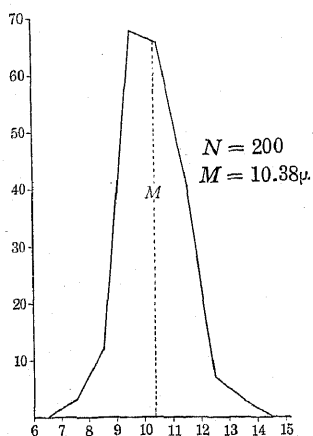


Abb. 2. Die Variationskurve in bezug auf die Länge des Kernstückes.

Länge d. Kernstückes (μ)	7	8	9	10	11	12	13	14
Frequenz	3	12	68	66	41	7	3	

gewöhnlicher Länge und die hufeisenförmigen Kernstücke nach Fixierung und Färbung verneinen aber diese Vermutung und zeigen, daß sie reif sind. Weiter konnte ich einige mißgebildete Spermatozoiden, deren Kernstücke wie bei dem Kernstück des mißgebildeten Spermatozoids von *Dumortiera* an ihrem hinteren Ende zweifach gabelt sind, beobachten (Abb. 12). Das Ausmaß der Gabelung ist verschieden; man kann Exemplare von einer dem normalen Typus sehr nahe stehenden Stufe bis zu der, an welcher zwischen vorderem und hinterem Ende nicht zu unterscheiden ist, sehen. Abb. 3 zeigt schematisch diese verschiedene

Ausmaß der mißgebildeten Spermatozoiden.

Das Plasmastück haftet gewöhnlich am hinteren Ende des Kernstückes an. Es ist nach dem Berichte Mühldorf's (1930) „endständig“. Seine genaue Stellung, Form und Größe sind nicht bestimmt wie bei *Wiesnerella* (Miduno 1935). Das Plasmastück färbt sich mit Gentianaviolett schwach, und im inneren Teil desselben wurden einige dunkel gefärbte Punkte erkannt, ihre Zahl und Form aber sind unbeständig. Nicht alle

Spermatozoiden haben ein Plasmastück, sondern man kann auch viele Spermatozoiden ohne Plasmastück finden. Vermutlich haben sie die Plasmastücke während des Schwimmens verlassen, da bei dem unreif ejakulierten Spermatozoiden das Plasmastück immer beobachtet wurde.



Abb. 3. Die normale (a) und die mißgebildeten Formen (b-g) des Spermatozoids in zunehmendem Grad. ca. 4800 ×.



Die Stammsubstanz stößt an das vordere Ende des Kernstückes. Sie scheint sehr gebrechlich zu sein, da die Form derselben bei dem Strichpräparat unbeständig ist. Abb. 4 zeigt die Form, welche am häufigsten beobachtet wird. Die Stammsubstanz färbt sich mit Gentianaviolett schwach. Die Grenzlinie zwischen dem Kernstück und der Stammsubstanz ist klar und man kann oft die getrennte Stammsubstanz finden (Abb. 9). An der besprochenen Art kann man die Basalkörner erkennen, aber nicht die Spitzensubstanz, welche in *Wiesnerella* beobachtet wurde (Miduno 1935).

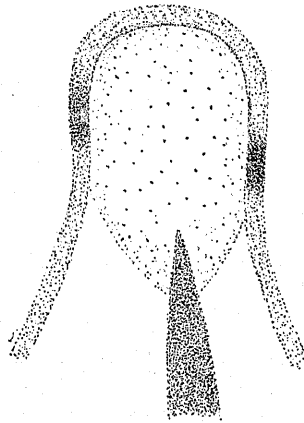


Abb. 4. Der vordere Teil des Spermatozoids. Von oben gesehen. (Schematisch gezeichnet und stark vergrößert.)

Die Zilien entstehen von den Basalkörnern in der Stammsubstanz wie die Spermatozoiden der anderen Lebermoosarten. Die Zahl der Zilien ist zwei. Die Länge der beiden Zilien ist ungleich, und die Differenz zwischen der Länge beider beträgt gewöhnlich  $5\ \mu$ . Dieser Unterschied ist zu groß, daß man ihm als Messfehler ansehen könnte. Abb. 5 und 6, die nach der Messung von etwa 200 Spermatozoiden gezeichnet wurden, zeigen die Variationskurve und die Mittelwerte für die längeren und kürzeren Zilien:  $34.94\ \mu$  und  $29.85\ \mu$ . Das Verhältnis zwischen Zilienlänge, welche aus dem Durchschnitt beider Zilienlängen erhalten wurde, und Körperlänge (Länge des Kernstückes) ist 3. Diese Zahl ist die größte unter den bisher vom Verfasser beobachteten Lebermoosarten und ein wichtiges Merkmal für diese Art. Demgemäß kann man verstehen, daß beim Spermatozoid dieser Art die Zilie im Verhältnis zum Kernstück lang ist. Die Zilien-

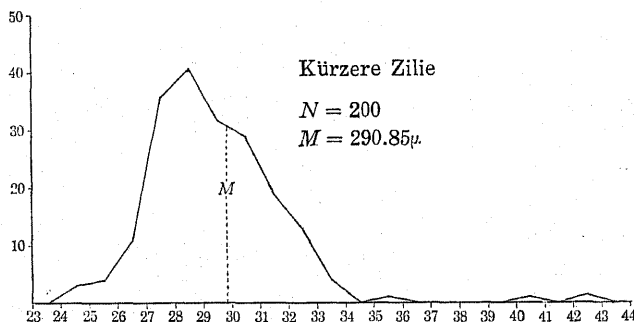


Abb. 5. Die Variationskurve in Bezug auf die Länge der kürzeren Zilie.

Länge der Zilie ( $\mu$ )	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
Frequenz	3	4	11	36	41	32	34	19	13	4	0	1	0	0	0	0	0	1	1

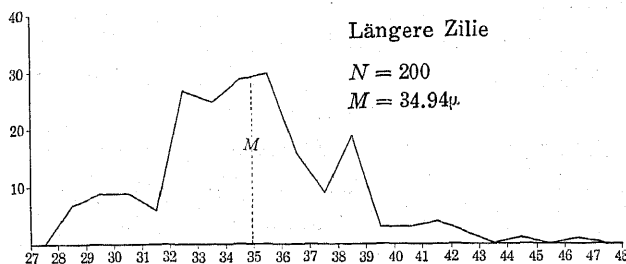


Abb. 6. Die Variationskurve in Bezug auf die Länge der längeren Zilie.

Länge der Zilie (μ)	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47
Frequenz	7	9	9	9	6	27	25	29	30	16	9	19	3	3	4	2	0	1	0	1

länge der mißgebildeten Spermatozoiden oder Zwergspermatozoiden ist gleich derjenigen der normalen Spermatozoiden, und der Mittelwert der Zilienlänge einiger mißgebildeter Individuen zeigt  $36.6 \mu$ .

Tabelle 1. Das Verhältnis zwischen Zilienlänge und Körperlänge des normalen Spermatozoids und des Zwergspermatozoids

	Normales Spermatozoid	Zwergspermatozoid
Zilienlänge	3	5
Körperlänge		

Folglich ist das Verhältnis zwischen Zilienlänge und Körperlänge erstaunlich groß, nämlich 5 (Tabelle 1). Diese Tatsache zeigt die Beziehungen zwischen dem Kernstück und der Zilie während ihres Entwicklungsprozesses, nämlich daß selbst wenn das Kernstück sich mißgestaltig entwickelt, die Zilien doch normal wachsen.

Vermutlich stellt diese Erscheinung ein interessantes Problem in Bezug auf Blepharoplast dar. Ausnahmsweise konnte der Verfasser in den zahllosen Spermatozoiden fünf Exemplare mit drei Zilien finden (Abb. 10). Seit Schowalter (1926) in *Riccardia pinguis* ein Spermatozoid mit drei Zilien gefunden hat, hat der Verfasser in drei Lebermoosarten, nämlich in *Dumortiera* (Miduno 1934), *Wiesnerella* (Miduno 1935) und jetzt in *Reboulia*, Spermatozoiden mit mehr als zwei Zilien beobachtet. Folglich gibt es jetzt unter den Lebermoosen vier Arten, einige deren Spermatozoiden mit mehr als zwei Zilien versehen sind. Bei der besprochenen Art ist die Stammsubstanz sehr kurz, und es war sehr schwer, die Entfernung der Basalkörner sogar bei dem Spermatozoid mit zwei Zilien klar zu sehen. Da Spermatozoiden mit mehr als zwei Zilien zu wenig gefunden worden waren, erwies es sich als unmöglich zu bestimmen, ob es bei solchen Spermatozoiden zwei oder mehr Basalkörner gibt. Die Zilienlänge der mit drei Zilien versehenen fünf Spermatozoiden ist wie in Tabelle 2 gezeigt. Die Spitze der Zilie gabelt sich in zwei Teile, wenn die

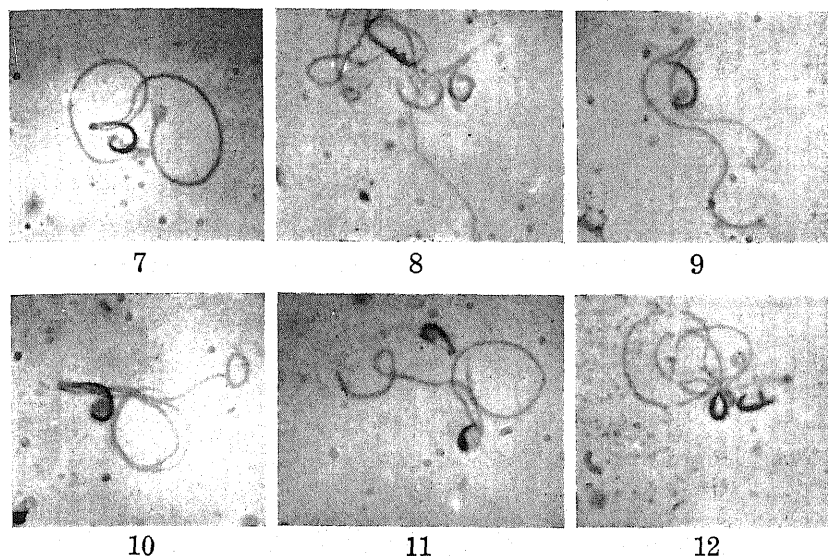


Abb. 7-12. ca. 1200 $\times$ . 7. Ein normales Spermatozoid mit zwei Zilien. Man kann die gegabelte Spitze der Zilien sehr deutlich sehen. 8. Das Spermatozoid zeigt die Basalkörner ziemlich klar. 9. Die getrennte Stammsubstanz und das Kernstück. 10. Das Spermatozoid mit drei Zilien. 11. Das Zwergspermatozoid. 12. Das mißgebildete Spermatozoid. Ende des Kernstückes ist zweifach gegabelt.

beiden Zinken auch nicht immer geöffnet sind, und zwischen beiden Zinken kann man die Plasmasubstanz erkennen, welche mit Gentianaviolett schwach gefärbt wird (Abb. 7).

Tabelle 2. Der Zilienlänge der mit drei Zilien versehenen fünf Spermatozoiden

Spermatozoid	Kernstück	1. Zilie	2. Zilie	3. Zilie	Gesamtsumme
Nr. 1	10 $\mu$	25 $\mu$	26 $\mu$	26 $\mu$	77 $\mu$
Nr. 2	9	26	27	32	85
Nr. 3	9	24	27	35	86
Nr. 4	10	27	29	30	86
Nr. 5	10	29	33	33	95
Mittelwert	9.6	26.2	28.4	31.2	85.8

Tabelle 3. Die Morphologie des jeden Teils von *Reboulia*-Spermatozoid

Art	Körper (Kernstück)					Geißel				Geißel- länge	Form des Plasma- stückes
	From	Länge	Dicke	vor- deres Ende	hin- teres Ende	Zahl	Länge		Spitze	Körper- länge	
							län- gere	kür- zere			
<i>Rebou- lia hemi- sphae- rica</i> (L.) RADDI.	hufe- sen- förmig	10.38 $\mu$	ca. 0.65 $\mu$	zuge- spitzt	zuge- spitzt	2 sel- tens 3	34.94 $\mu$	29.85 $\mu$	gega- belt	3.1	unbe- ständig

Die oben erwähnten Merkmale des Spermatozoids der in Betracht kommenden Art wurden in der Tabelle 3 wiedergegeben.

### Zusammenfassung

1) Das Kernstück ist hufeisenförmig oder sichelförmig, und die Länge desselben ist  $10.38 \mu$ . Ausnahmsweise wurden Kernstücke, die  $2/3$  so lang wie die normalen sind, gefunden. Auch Kernstücke, deren beide Enden in verschiedenem Ausmaße zweifach gegabelt sind, wurden gefunden.

2) Das Plasmastück der reifen und schwimmenden Spermatozoiden war nicht immer zu finden. Die Form des Plasmastückes ist unbeständig.

3) Die Zahl der Zilien ist gewöhnlich zwei. Als Ausnahme wurden fünf Spermatozoiden mit drei Zilien gefunden.

4) Bei *Reboulia hemisphaerica* sind die Zilien im Verhältnis zu den Kernstücken lang. Das Verhältnis zwischen diesen beiden ist 3, demgemäß ist die Zahl bei weitem größer als bei *Marchantia*, *Conocephalus*, *Dumortiera*, oder *Wiesnerella*. Darin findet man also ein auffallendes Merkmal für diese Art.

5) Bei dem Zwergspermatozoid ist die Zilienlänge gleich der des normalen Spermatozoids, und das Verhältnis zwischen Zilienlänge und Körperlänge wird ungewöhnlich groß, nämlich 5. Hieraus kann man ein wichtiges Problem in bezug auf das Blepharoplast erkennen.

Zum Schluß sei es mir gestattet, meinem hochverehrten Lehrer, Herrn Dr. Y. Sinotô, nach dessen wohlwollendem Vorschlag ich diese Forschung machte, an dieser Stelle meinen herzlichsten Dank auszusprechen.

Ein Teil der Ausgaben für diese Arbeit ist durch Gewähren einer Stiftung seitens der Japanischen Gesellschaft zur Förderung wissenschaftlicher Forschungen getragen worden, wofür der Verfasser hiermit seinen verbindlichsten Dank ausdrückt.

Genetik-Abteilung, Bot. Inst., Naturwissenschaftl. Fakultät d. Kaiserl. Universität Tokyo

### Literaturverzeichnis

- Miduno, T. 1934. Zytologische Untersuchungen der Bryophyten, I. Die Morphologie der Spermatozoiden einiger Hepaticen. Jour. Fac. Sci., Imp. Univ. Tokyo, Sect. 3. Bot. 4: 367-387.
- 1934. Zytologische Untersuchungen der Bryophyten, II. Die Morphologie des Spermatozoids von *Wiesnerella denudata*. Bot. Mag. (Tokyo) 48: 814-822.
- Mühdorf, A. 1930. Berichtigungen und Ergänzungen unserer Kenntnisse über die Morphologie und Histologie pflanzlicher Spermien. Biol. Gen. 6: 457-482.
- Schowalter, A. M. 1926. Studies in the cytology of the Anacrogynae. I. Antherozoids. Ann. Bot. 40: 691-708.

## The Discovery of the Relation Between the Nucleolus and the Chromosomes

By

Prof. R. Ruggles Gates

The last decade has seen a striking advance in our understanding of the origin of the nucleolus in the mitotic cycle and its relation to the chromosomes. In tracing briefly the history of this development, which represents a major discovery in relation to the organization of the nucleus as a whole, we may treat the subject as an instance of the methodology of science; for it illustrates how a slight increment of knowledge may lead to a complete change or reversal in the point of view. In this connection it will be necessary to refer only to some of the more essential papers, and a full consideration of the literature will not be attempted.

Early cytological observers frequently noted some connection between the nucleolus of plant cells and the network of the nucleus. For example, Wager (1904), in the root tip cells of *Phaseolus*, regarded the nucleolus as suspended in the "nuclear network" by numerous fine threads. He concluded that "the nucleolus simply forms part of the nuclear network", and believed it to be concerned in prophase with the formation of the chromosomes. Strasburger had previously conjectured that it played some part in the formation of the spindle. At a still earlier period, Farmer (1895) had concluded from many observations on the spore mother cells of various Liverworts that the nucleolus was associated with the chromosomes by means of delicate threads "in an unmistakable and remarkable manner", and he was inclined to support the view that some of the nucleolar material was passed into the chromosomes.

The first evidence of a definite morphological relation between nucleolus and chromosomes came much later and was contained in a paper by Latter (1926) on the pollen development of *Lathyrus odoratus*. By careful destaining, she was able to demonstrate in the nucleolus of the pollen mother cells during the meiotic prophase a more deeply staining and peripherally placed area to which she gave the name nucleolar body. This body was seen to be present and conspicuous in the leptotene, zygotene and pachytene stages of the nucleus, it frequently projected as a papillate or lens-shaped structure from the surface of the nucleolus and, most significantly, it was found throughout the thread stages to be constantly attached to the

chromatin thread, one loop of the "continuous spireme" always passing through this body. An attachment, which was obviously of morphological significance, was thus established between the chromatin thread and the nucleolus in prophase.

In a series of further papers from this Laboratory, such a fixed relationship was soon established in the pollen mother cells of a number of plants. Sheffield (1927) and Gates and Sheffield (1929), showed it in *Oenothera*; Gates and Latter (1927) in *Lathraea*, where, in some cells, there were two such attachments instead of one; Latter (1932) in *Malva sylvestris*; Selim (1930) in rice, where, in some varieties, two nucleoli were usually found in contact, with a loop of the thread attached at their point of contact. These observations were confined to pollen mother cells.

As we have seen, it was frequently assumed in the early literature that the nucleolus gave up some of its material to the "spireme" either directly or indirectly, and that there was a flow of chromatin along the thread. Fikry (1930) subjected all such theories to a stringent criticism and pointed out the difficulties which they involved.

Another line of observations which led towards the present point of view began with the discovery of satellites or trabants on the chromosomes of *Galtonia* by S. Navashin (1912). He was led to conclude, from studies of somatic prophase, that the satellites were at first attached to the nucleolus, while the body of a certain pair of chromosomes at an advanced stage of condensation moved up to the nucleolus and drew the satellites away. Baranov (1926) found in *Drimiopsis maculata* ( $2n = 64$ ) that the satellites were of two sizes, 4 large ones attached to the long chromosomes and 12-16 small ones belonging to the short chromosomes. In prophase these satellites were all attached to the nucleolus and were believed to be lifted off later by their respective chromosomes. Similar conditions were seen at the leptotene stage and at synizesis. Probably in all such cases the delicate thread connecting the prophase chromosome to its satellite was broken in the process of fixation. Navashin was correct in finding that a pair of satellites were associated with the nucleolus, but much later evidence shows that they generally remain attached to the rest of the chromosome by a delicate thread of varying length. Smith (1933) showed in *Galtonia* that the satellites remain attached to their respective chromosomes in prophase while generally remaining in contact with the nucleolus. He also found that in meiosis the two satellite chromosomes form a bivalent whose satellites are independently attached to the nucleolus, sometimes with a constriction or bud of the nucleolus at the attachment region. No fixed differences in size of satellites were found in this material.



Satellites are now a familiar feature in the morphology of plant and animal chromosomes. The papers concerned with them are much too numerous to enumerate here, but references will be made to a few papers to indicate their range of occurrence and variation in structure.

S. Navashin (1927) found an "asymmetrical" race of *Galtonia*, having a large and a small satellite attached to the nucleolus in root-tip nuclei. Similar conditions have since been found in a number of other plant genera. In tetraploid cells with resting nuclei he figures two large and two small satellites attached to the nucleolus. Taylor (1926) found a single plant of *Allium* in which the root-tip cells contained, instead of a pair of J-shaped chromosomes each with a satellite, one such chromosome having two satellites arranged tandem. It is not clear what has happened in this case. The duplication of the satellite may have been through translocation from its homologue.

Philp and Huskins (1931) concluded from a study of the ever-sporting races of *Matthiola incana* that they have a heteromorphic pair of (A) chromosomes, one of the members having lost a satellite, pure singles and doubles having no such loss. These results have, however, been disputed (Westergard, 1936) and are therefore in doubt.

De Mol (1926), from an examination of seedlings of *Hyacinthus*, found that the number of nucleoli in the cells was a reliable guide to the polyploidy of the plant, diploids having generally two nucleoli, triploids three and tetraploids four. Moreover, the simple nucleoli were all of the same size, the complex or fusion nucleoli when they occurred being larger. Kuhn (1928) gives a list of plants with satellite-chromosomes. He examined 37 species of *Thalictrum* and found that all have trabants, and he concluded that univalent idiograms apparently never have more than one pair.

Heitz apparently deserves the credit for recognizing the true relation between nucleolus and chromosomes. He studied (1931a) the somatic mitoses in a number of plants, including 9 Dicotyledons, 2 Monocotyledons and one Liverwort, and from these observations concluded that the nucleoli arise in telophase from the satellites on a certain chromosome pair, the two nucleoli fusing to form one if the members of the pair were near enough together to bring the nucleoli into contact. In another paper (Heitz, 1931b), he examined 33 species of *Vicia*, finding two or four satellited chromosomes normally present in each. He concluded that all plants probably have satellited chromosomes which give rise to the nucleoli in telophase, but he found that a single lagging chromosome which has no satellite may nevertheless produce a small nucleus containing a nucleolus.



In the same year McClintock (1931) published figures and photographs of the pollen mother cell nuclei of Maize showing the zygonema attached to the nucleolus, but without reference to the earlier work on this subject. Further photographs showing the same relationship incidentally to a study of zygotene stages were published later (1933), and the satellited chromosome pair in maize is now known to be chromosome VI. McClintock (1934) studied in detail a case of reciprocal translocation between chromosomes VI and IX in maize. She called the deeply staining body at the point where the sat.-chromosome is attached to the nucleolus the nucleolar-organizing body. In the case of translocation studied, the break had occurred at this body, which was thus divided into two unequal parts, both interchanged chromosomes possessing a portion. Each portion developed a nucleolus, so that plants homozygous for the interchange developed four nucleoli in their somatic telophase while those heterozygous for it developed three. The nucleolar-organizing body of McClintock is evidently essentially the same element as the nucleolar body of Latter (1926). From other observations McClintock concluded that the number of nucleoli in somatic telophases is correlated with the number of sat.-chromosomes, haploid cells having one, diploids two and triploids three. It was also found that certain genomic deficiencies in maize interfere with the nucleolar body and lead to the formation of many small scattered nucleoli. It is concluded that these nucleoli are formed by the collection into droplets of the matrix of the chromosome.

The nucleolar body then appears to act normally as an organizing centre at one locus of a particular chromosome pair, apparently controlling the disposal of the matrix material of the chromosome which is given up in telophase and may be, at least in part, distributed to the chromosomes again in the following prophase.

Heitz (1933 a, b) has shown that in various species of *Drosophila* the X- and Y-chromosomes are attached to the nucleolus in prophase, the Y having a visible satellite and being composed almost entirely of deeply-staining heterochromatin (non-genic) while the proximal half of the X-chromosome of *D. melanogaster* is heterochromatic and the distal half euchromatic (pale-staining). The relationships of the nucleolus and chromosomes in the salivary gland nuclei in several species of *Drosophila* have recently been investigated by Frolova (1936), who finds a definite body, the chromocentre, in the nucleus, to which the proximal ends of all the chromosomes are attached. In addition, a fine spiral thread which stains with Feulgen attaches the chromocentre to the nucleolus, and has a dendritic ending in the nucleolus. The attachment is therefore

regarded as between the chromocentre and both the nucleolus and the X-chromosome, rather than directly between the nucleolus and the X. The origin of the chromocentre in the salivary glands is unknown, but it is suggested that it may arise from the union of the Leitkörperchen, or centromeres of all the chromosomes.

That the nucleolus arises at telophase in animal cells in essentially the same manner as in plant cells is shown by the careful investigation of Dearing (1934) on *Amblystoma*. He finds in the somatic cells a pair of chromosomes with rather large satellites. The history of these chromosomes is traced through mitosis and interkinesis. Each telophase chromosome consists of two chromatids, the satellite-chromosomes usually lying far apart so that two nucleoli are almost invariably present in the resting nuclei. Each nucleolus first appears in mid-telophase as two subterminal deeply-staining granules on the split chromosomes. These granules enlarge and fuse, the fusion body growing greatly in size and forming the nucleolus with the terminal satellite attached to it. In prophase the two nucleoli remain attached to their respective chromosomes, gradually diminishing in size until they finally disappear, leaving only the thread which attaches the satellite to the chromosome.

In one larva all the epithelial cells contained three nucleoli, although the chromosome number (28) remained the same. The extra nucleolus occupied a *central* position in one of the arms of a long U-shaped chromosome. This is an exception to the rule that the nucleolar body is subterminal in position, and also to the rule that an extra nucleolus generally indicates an extra genome in the cell.

Isamu Stow (1935) finds a correlation between the presence of a satellite and the leaf shape in the genus *Paris*. In *Paris* and *Trillium* four of the five chromosomes are found to be homologous from species to species, but in the genus *Paris* the fifth differs from species to species. It is found that in *Paris*, as in *Galtonia* and *Muscari*, the loss of a satellite from one chromosome gives a plant of more vigorous growth. There is also in *Paris* a change in the length-breadth index of the leaves, the breadth being greater in plants with a single satellite instead of two. In a study of the satellites of *Narcissus*, Fernandes (1935) claims to have shown wide variation in the size of satellites in the same plant, every transition appearing between (1) a body half as large as a chromosome arm, (2) a very small satellite, (3) a filament only and (4) complete absence of any trace in a particular pair of chromosomes. He therefore doubts the results of M. Navashin (1926), who found in *Crepis Dioscoridis* three types of plants having respectively two, one or no satellite in the ratio 43:90:42, the last type being non-viable and recognizable only

in the cell divisions of the embryos. It is well known that the apparent size of satellites varies with the depth of staining, and it appears probable that the variable results of Fernandes are due to insufficient control of this factor. In a series of interspecific hybrids of *Crepis*, M. Navashin (1927) found that in certain crosses a particular satellite disappeared. This he termed amphiplasty. The author (1934) distinguishes between differential amphiplasty, which affects individual chromosomes, and neutral amphiplasty, which affects the whole genome. Disappearance of the satellite from one chromosome has been described in 13 different interspecific *Crepis* hybrids, always due to its fusion with the proximal end of the satellited chromosome. It appears, therefore, that it is the connecting thread which is really suppressed. The meaning of this and its relation to nucleolar formation requires further investigation.

In a further study of the satellites in *Narcissus reflexus* and *N. bulbocodium*, Fernandes (1936) refers to the region of the chromosome which elaborates the nucleolus as the nucleologenic region. He finds that in *N. bulbocodium* L. var. *genuinus* around Coimbra there are no satelliferous chromosomes, but one pair of chromosomes has terminal heterochromatic (deep-staining) corpuscles which attach them to the nucleolus in prophase. This suggests a resemblance in prophase to the condition in rice (see below). In the prophase of mitosis in the pollen grain of this type Fernandes finds some grains with two chromosomes attached to the nucleolus and some with one, from which he concludes that in the somatic cells of this type three nucleolus-forming chromosomes are present. This may resemble the exceptional condition found by Dearing (1934) in *Amblystoma*, but the position is not clear. Lorbeer (1934) has also found in liverworts (*Frullania*) that the nucleolar chromosomes are attached by their heterochromatic extremities to the nucleolus and have no filament or satellite. In *Sphaerocarpus Donnellii* he figures the heteropyknotic X- and Y-chromosome attached to the nucleolus in prophase, while another pair of chromosomes bear the satellite. Bruun (1932), in his comparative survey of the chromosomes in the genus *Primula*, finds no satellites in *P. seclusa*, although they are present in the related species. This is probably another case where the nucleolus-producing pair of chromosomes have no filament or the nucleolar body is terminal.

In a recent paper Nandi (1937a) has investigated more fully the relation of nucleolus to satellites in the pollen mother cells of *Oryza*. He confirms the finding of Selim (1930) that when two nucleoli are present the zygotene thread is always attached at their point of contact. When a single nucleolus is present at zygotene a

pair of threads (uncondensed chromosomes) are attached to it, each by a terminal knob. In other words, there is no delicate thread between the satellite and the rest of the chromosome, and the nucleolus is presumably produced directly by the terminal knob or satellite. In various other plants, where the nucleolar body is subterminal, the nucleolus as it grows must lengthen or stretch this intervening thread, for the satellite often lies on one side of the mature nucleolus and the body of the chromosome on the other, the thread lying over the surface of the nucleolus and generally remaining intact between them. At diplonema in rice the knobbed bivalent remains attached to the nucleolus, and this relationship continues into late diakinesis, when the bivalents are fully condensed and even after the nucleolus has greatly diminished in size preparatory to its complete disappearance. In somatic chromosomes, Nandi (1937b unpublished) found two pairs with satellites, supporting incidentally the view that *Oryza sativa* is a secondary tetraploid; but as mentioned above, only one pair was found attached to the nucleolus in pollen mother cells. The terminal knob in this case may possibly represent the nucleolar body, the very minute satellite and its thread having become invisible. Hedayetullah (1933) has found that the Indian variety of rice, "Kochivittu", which has two large nucleoli of equal size (Selim, 1930) when crossed with "Nabatat", an Egyptian variety with one large nucleolus, or with "White Aus", a variety with two unequal nucleoli, gives hybrids with two unequal nucleoli in the pollen mother cells.

It appears probable that there are two kinds of satellites; (1) those, generally larger, in connection with which the nucleolus arises. These are generally one pair, and when more than one this is probably due to primary or secondary polyploidy. (2) Those, generally smaller, which, in such plants as *Drimiopsis maculata*, do not take direct part in nucleolus formation. It is probable that in some cases where the spindle fibre attachment is subterminal the Leitchkörperchen or centromere has been mistaken for a true satellite.

### Summary

The discovery that a particular pair of satellited chromosomes is concerned in producing the nucleolus in telophase depended on several independent lines of observation. The satellites were first observed by S. Navashin in 1912 as bodies attached to the nucleolus, and were believed to become attached to their respective chromosomes later in the prophase.

The earlier cytologists believed the nucleolus to arise as an independent body in telophase and to pass material on to the chro-

matin thread in prophase. A definite connection of morphological significance was first observed in pollen mother cells of *Lathyrus* by Latter in 1926, between the nucleolus and the chromatin thread, the latter being constantly attached in leptotene and zygotene stages to a more deeply staining portion of the nucleolus which was called the nucleolar body.

In 1931, from observations of somatic telophase in various plants, Heitz concluded that a particular pair of satellited chromosomes was responsible for producing two nucleoli, or one if they were lying near enough together to touch and fuse.

In 1934, from a reciprocal translocation in maize in which the nucleolar body was broken into two, McClintock concluded that the nucleolar body was situated at a particular locus of the chromosome adjacent to the satellite, and that this body was normally concerned in organizing the nucleolus from the material of the chromosome matrix.

In 1934, essentially the same relationships were shown for animals by Dearing, through an investigation of the somatic nuclei of *Amblystoma*. Two subterminal granules on the split telophase chromosome were found to fuse shortly, afterwards growing gradually into a nucleolus from that chromosome, which bears a large satellite at metaphase.

Among subsequent observations, it is found that in the pollen mother cells of rice a terminal knob rather than a satellite is attached to the nucleolus, and that this attachment remains while the nucleolus gradually disappears. *Primula seclusa* and certain strains of *Narcissus bulbocodium* var. *genuinus* near Coimbra also appear to lack satellited chromosomes, yet produce a nucleolus. Similar conditions were found in Liverworts. The presence of a connecting thread is therefore unessential.

A diploid nucleus in plants or animals then usually contains a pair of chromosomes which are mainly concerned in organizing the nucleolus in telophase at a particular locus. This locus is usually subterminal, giving a satellite attached by a thread, which may increase greatly in length as the nucleolus grows.

Various other conditions are derivable from this through translocation, or perhaps in some cases through mutation. Questions of variation in the size of satellites, or their disappearance, and their relation to particular phenotypic characters require further elucidation.

There appear to be two kinds of satellites, one concerned in the production of a nucleolus and the other not. Why the nucleolar body should be generally subterminal on a chromosome is not clear, nor

is the nature of the delicate thread connecting the satellite to the chromosome.

#### References

- Baranov, P. 1926. Cytologische und embryologische Untersuchungen an *Drimiopsis maculata* Lindl. Zeits. f. Zellforsch. mikr. Anat. 3: 131-148, figs. 28.
- Bruun, H. G. 1932. Cytological studies in *Primula*, with special reference to the relation between the karyology and taxonomy of the genus. Symb. Bot. Upsalienses 1: 3-239, figs. 37.
- Dearing, W. H. Jr. 1934. The material continuity and individuality of the somatic chromosomes of *Amblystoma tigrinum* with special reference to the nucleolus as a chromosomal component. Journ. Morphology 56: 157-174, pls. 3.
- Farmer, J. B. 1895. On spore-formation and nuclear division in the Hepaticae. Ann. of Botany 9: 469-523, pls. 3.
- Fernandes, A. 1935. Les satellites chez *Narcissus reflexus* Brot. et *N. triandrus* L. I. Les satellites des métaphases somatiques. Bol. d. Soc. Broteriana 10: pp. 33, figs. 39.
- 1936. Les Satellites chez les Narcisses. II. Les satellites pendant la mitose. Bol. Soc. Broteriana 11: 87-146, figs. 24, pls. 2.
- Fikry, M. A. 1930. Phenomena of heterotypic division in the pollen mother-cells of a tetraploid form of *Rumex scutatus* var. *typicus*. Journ. Roy. Micros. Soc. 50: 387-419, pls. 6.
- Frolova, S. L. 1936. Struktur der Kerne in den Speicheldrüsen einiger *Drosophila*-Arten. Zytol. Abt. Inst. exp. Biol. Moskau 5: 271-292, figs. 25.
- Gates, R. R. and Latter, J. 1927. Observations on the pollen development of two species of *Lathraea*. Journ. Roy. Micros. Soc. 47: 209-225, figs., 1, pls. 6.
- Gates, R. R. and Sheffield, F. M. L. 1929. Megaspore development in *Oenothera rubricalyx*, with a note on Chromosome linkage in *Oenothera angustissima*. Proc. Roy. Soc. B. 105: 499-517, pls. 3.
- Hedayetullah, S. 1933. A cytogenetical study of the behaviour of nucleoli in the pollen mother cells of three varieties of rice (*Oryza sativa* L.) in the parent plants and in their hybrids. 20th Ann. Meet. Indian Sci. Congr. Patna (cited in Plant Breeding Abst. 4: 11, 1934).
- Heitz, E. 1931a. Nukleolen und Chromosomen in der Gattung *Vicia*. Planta, Arch. f. wissens. Bot. 15: 495-505, figs. 2.
- 1931b. Die Ursache der Gesetzmäßigen Zahl, Lage, Form und Größe pflanzlichen Nukleolen. Planta 12: 775-844, pls. 2, figs. 26.
- 1933. Über totale und partielle somatische Heteropyknose, sowie strukturelle Geschlechtschromosomen bei *Drosophila funebris*. Zeits. f. Zellforsch. u. mikros. Anat. 19: 720-742, figs. 12.
- Kuhn, E. 1928. Zur Zytologie von *Thalictrum*. Jahrb. Wiss. Bot. 68: 382-430, figs. 14, pl. 12.
- Latter, J. 1926. The pollen development of *Lathyrus odoratus*. Ann. Botany 40: 277-313, pls. 3.
- 1932. The meiotic divisions in the pollen mother-cells of *Malva sylvestris*. Ann. Botany 46: 1-10, pls. 2.
- Lorbeer, G. 1934. Die Zytologie der Lebermoose mit besonderer Berücksichtigung allgemeiner Chromosomenfragen. Jahrb. Wiss. Bot. 80: 567-818, figs. 110, pl. 1.
- McClintock, B. 1931. Cytological observations of deficiencies involving known genes, translocations and an inversion in *Zea mays*. Missouri Agric. Exper. Stn. Research Bull. No. 163, pp. 30, figs. 39.
- 1933. The association of non-homologous parts of chromosomes in the mid-prophase of meiosis in *Zea mays*. Zeits. f. Zellforsch. u. mikros. Anat. 19: 191-237, figs. 51, pls. 6.
- 1934. The relation of a particular chromosomal element to the development of the nucleoli in *Zea mays*. Zeits. f. Zellforsch. u. mikros. Anat. 21: 294-328, figs. 21, pls. 7.



- de Mol, W. E. 1926. Nucleolar number and size in diploid, triploid and aneuploid Hyacinths. *La Cellule* 38: 7-64, pls. 3.
- Nandi, H. K. 1937 a. Cytological investigations of rice varieties. *Cytologia* (in the press).
- 1937 b. *Journ. Genetics* (in the press).
- Navashin, M. 1926. Variabilität des Zellkerns bei *Crepis*-Arten in Bezug auf die Artbildung. *Zeits. f. Zellfors. u. mikros. Anat.* 4: 171-215, figs. 14.
- 1927. Über die Veränderung von Zahl und Form der Chromosomen infolge der Hybridisation. *Zeits. f. Zellfors. u. mikros. Anat.* 6: 195-233, figs. 15.
- 1934. Chromosome alterations caused by hybridization and their bearing upon certain general genetic problems. *Cytologia* 5: 169-203, figs. 5, pls. 1.
- Navashin, S. 1912. Sur le dimorphisme nucléaire des cellules somatiques de *Galtonia candicans*. *Bull. Acad. Imp. Sci. St. Petersburg* 6: 373-385.
- 1927. Zellkerndimorphismus bei *Galtonia candicans* Des. und einigen verwandten Monokotylen. *Ber. d. Deuts. Bot. Gesells.* 45: 415-428, pl. 1.
- Philp, J. and Huskins, C. L. 1931. The cytology of *Matthiola incana* R. Br. especially in relation to the inheritance of double flowers. *Journ. Genetics* 24: 359-404, pl. 1, figs. 63.
- Selim, A. G. 1930. A cytological study of *Oryza sativa* L. *Cytologia* 2: 1-26, figs. 47.
- Sheffield, F. M. L. 1927. Cytological studies of certain meiotic stages in *Oenothera*. *Ann. of Botany* 41: 779-816, figs. 3, pls. 3.
- Smith, F. H. 1933. The relation of the satellites to the nucleolus in *Galtonia candicans*. *Am. Journ. Bot.* 20: 188-195, pl. 1.
- Taylor, W. R. 1925. The chromosome morphology of *Veltheimia*, *Allium* and *Cyrtanthus*. *Am. Journ. Bot.* 12: 104-115, figs. 35.
- Wager, H. 1904. The nucleolus and nuclear division in the root-apex of *Phaseolus*. *Ann. Botany* 18: 29-55.

#### Note added in proof:

A similar origin of the nucleolus in connection with particular chromosomes has recently been found in Protozoa (T. T. Chen, *Proc. Nat. Acad. Sci.* 22: 602-7, 1936). In the opalinid ciliate, *Zelleriella intermedia*, which is diploid,  $2n = 24$  and the chromosomes show constant structural differences. Some races of *Z. intermedia* have 4 nucleoli and some 6. Each is formed on a separate chromosome and they are always non-terminal. These nucleoli were previously mistaken for massive chromosomes. They differ from those in other organisms in that they surround a portion of the chromosome, which is visible through the more transparent elongated nucleolus. In mitosis they do not disappear but divide and are carried to the poles. Thus the behaviour of the nucleolus in Protozoa differs in important respects from that in higher animals and plants.



## Les chromosomes du genre *Gryllotalpa* gryll. L.

par

H. de Winiwarter, Liège

(19 figures dans le texte)

En étudiant le genre *Mecostethus*, MCCLUNG a constaté chez trois représentants de ce groupe (*M. gracilis*, *grossus* et *lineatus*), une remarquable similitude de l'appareil chromosomal.—Non seulement le nombre des chromosomes est identique (23 chez le mâle, et 24 chez la femelle), mais leur forme et leur arrangement à la métaphase sont caractéristiques, au point que la simple inspection d'une plaque équatoriale permet de reconnaître l'espèce et d'affirmer qu'elle appartient au groupe *Mecostethus*. Cette identité se révèle d'ailleurs encore plus complète, lorsqu'on étudie chaque chromosome individuellement, car chez toutes les trois formes, on retrouve des chromosomes identifiables grâce à des signes particuliers, ce qui permet de les suivre durant la période d'accroissement et les mitoses de maturation. MCCLUNG les désigne sous les termes de "grand et petit anneau", "tétrade dimorphique" et "ditactic chromosome". Et il conclut de la similitude de ces éléments particuliers à l'identité probable de tous les autres chromosomes de la garniture.

Il en résulte que dans un groupe bien circonscrit tel que celui dont il s'agit, les espèces ne se ressemblent pas seulement par leurs caractères extérieurs, mais encore par la forme et l'organisation de leurs chromosomes. En d'autres termes, la constance de la structure cellulaire s'exprime parallèlement dans celle de la structure du corps.

Cette constatation est d'une grande importance générale. Si le matériel de MCCLUNG est particulièrement favorable pour une étude de ce genre, il est cependant nécessaire d'étendre ces investigations à d'autres groupes. Sur le propre conseil de MCCLUNG, j'ai repris le matériel de *Gryllotalpa* qui m'avait servi naguère à une étude du cycle chromosomique de races de provenances diverses; ce sont les résultats de cette étude que je me propose d'exposer brièvement dans cette note.

On sait que l'espèce *Gryllotalpa gryll.* L. comporte trois races actuellement bien connues, d'après leur habitat et leur formule chromosomiale: 1° une race septentrionale (Belgique, France, Allemagne et Italie du Nord), chez laquelle le mâle possède 12 chromosomes (10 autosomes et 2 hétérochromosomes).

2°) une race méridionale (Italie du Sud), dont la formule comprend chez le mâle 14 autosomes et 1 hétéro.

3°) enfin, une race roumaine (VOINOV), qui possède 17 chromosomes en moyenne, car cette race se distingue par une grande tendance aux variations numériques.

A côté de *Gryllotalpa gryll.* (= *vulgaris*), le genre renferme encore *Gr. borealis* et *G. africana* (à 23 chromosomes, soit 22 autosomes et un hétéro), dont l'aire de dispersion est beaucoup plus vaste.

*G. borealis* de l'Amérique du Nord est identique à la forme de l'Amérique du Sud, décrite autrefois comme une espèce à part. *G. borealis* est une espèce de petite taille, et frappe par la tête dont les yeux sont très proéminents. Quant à *Gr. africana*, elle habite non seulement l'Afrique, mais encore de larges territoires en Asie. Il ressort surtout des données actuelles que *Gr. borealis* et *africana* possèdent des caractères morphologiques suffisants pour justifier la constitution d'espèces réelles, distinctes de *Gr. gryllotalpa*.

Disons de suite que les trois races de *Gryllotalpa gryll.* ne diffèrent que par le nombre de chromosomes; mais aucun caractère distinctif externe ne permet de reconnaître une race donnée en l'absence de l'analyse microscopique. Tous les entomologistes à

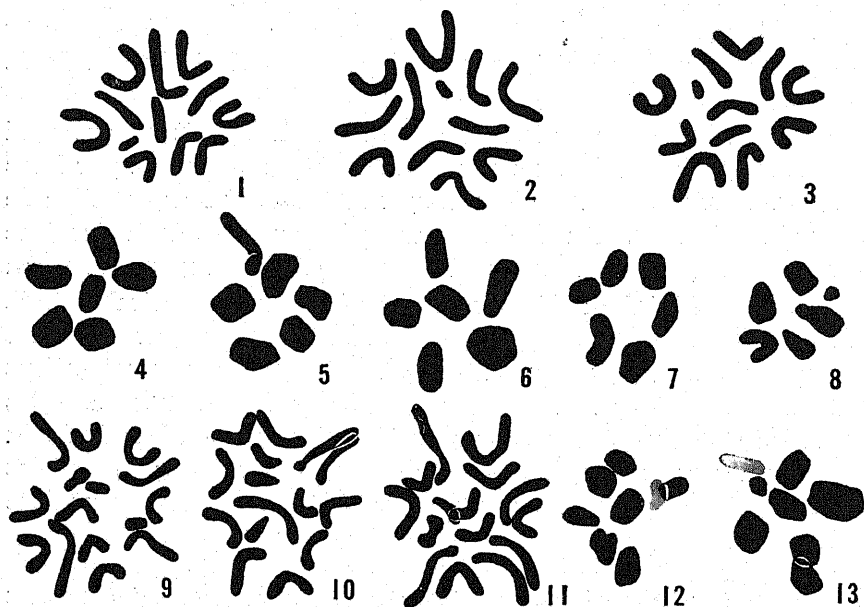


Fig. 1-13. *Gryllotalpa gryll.* L. race septentrionale: 1, 2 et 3 spermatogonies: 12 chr.—4, 5, 6, métaphases I, à 6 chrom.—7 et 8 métaphases II, à 6 chrom. (fig. 8. Y est le petit chromosome de droite).—race méridionale: 11, 12 et 13, spermatogonies à 15 chrom.—12 et 13, métaphases I, à 8 chrom. Le chromosome X en gris, sur un plan différent de celui des autosomes.— $\times 3500$ .

commencer par les plus autorisés (CHOPART, UVAROV, BARIGOZZI, etc.) sont d'accord pour considérer ces trois races comme appartenant à une seule et même espèce. C'est pourquoi j'ai préféré employer le terme de "race", plutôt que celui de variété ou de forme.

Je discuterai plus loin comment on peut concevoir cette différence numérique des chromosomes chez les trois races en question. J'examinerai d'abord les particularités ou les ressemblances que l'on relève sur les plaques équatoriales, dans la forme des chromosomes et leur évolution.

A. — *Gonies*. Dans les trois races (Cf. fig. 1 à 3, 9 à 11 et les fig. de VOINOV), les chromosomes sont bien étalés, mais ne constituent jamais une véritable couronne autour du fuseau central (Cf. les fig. 1 à 3 de MCCLUNG). Cette disposition est encore plus frappante chez *Gryll. borealis* (fig. 14 & 15) où le nombre de chromosomes est deux

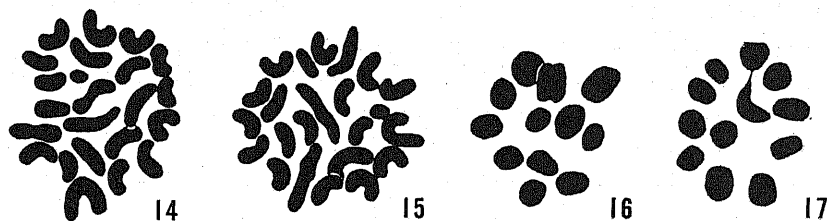


Fig. 14-17. *Gryllotalpa borealis*, d'après PAYNE (1912); 14 et 15 spermatogonies à 23 chrom.—16 et 17 métaphases I, à 12 chrom.  $\times$ ?

fois plus élevé. On pourrait objecter que ces figures se rapportent non à des métaphases proprement dites, mais à un stade pré-équatorial par exemple, tel qu'il se manifeste immédiatement après la disparition de la membrane nucléaire et la libération des chromosomes. Mais comme toutes les plaques équatoriales se présentent toujours sous l'aspect qui vient d'être décrit, il faut bien admettre qu'il s'agit de métaphases véritables, et que cette disposition est caractéristique pour l'espèce *Gryllotalpa*.

B. — *Forme des chromosomes de la gonie*. On retrouve régulièrement trois types de chromosomes: fers à cheval à branches égales ou inégales; crochets; bâtonnets plus ou moins longs. Les mêmes formes ont été décrites et figurées par d'autres auteurs, (PAYNE, VOINOV, BARIGOZZI), et le choix des couples homologues, effectué par BARIGOZZI et moi-même, aboutit à des séries absolument superposables. Il ne peut donc être question de coïncidences, et il faut admettre une constance parfaite de forme, ainsi que de nombre relatif de chaque variété de chromosomes. Au contraire, par exemple, chez *Mecostethus*, tous les chromosomes de la gonie, sans exception, sont des bâtonnets dont seule la longueur diffère, et possédant tous une insertion télomitique.

De part et d'autres, les métaphases de la gonie présentent donc un aspect caractéristique qui, du point de vue cytologique, apparaît comme une constante, au même titre que les attributs externes, distinctifs de l'espèce.

C. — *Période d'accroissement*. A la télophase de la dernière division goniale, les noyaux sont petits et plus larges que hauts. Les chromosomes individuels échappent rapidement à l'observation, à l'exception du ou des hétérochromosomes. Qu'il s'agisse d'un X-Y (race septentrionale), ou d'un seul X (race méridionale), ces éléments se distinguent par leur forme massive et leurs bords plus réguliers, des autosomes en train de se résoudre en un réseau délicat et serré.

Insensiblement, ce réseau reconstitue des filaments grêles et compliqués (n. leptotènes); ces filaments d'abord onduleux, s'orientent ensuite, pour passer au stade synaptène et enfin au stade pachytène. Pendant cette transformation, le volume du noyau augmente et les filaments se desserrent peu à peu. Après la centrotaxie, les gros filaments remplissent à nouveau la cavité nucléaire.

Durant toute cette évolution dont les aspects sont absolument identiques aux formes nucléaires de l'accroissement que j'ai sériés et décrits le premier, le seul élément identifiable est l'hétérochromosome. Le couple X-Y est plus long que l'X isolé, et généralement la composition double se révèle par une encoche qui partage l'élément en deux portions inégales. Ordinairement l'hétérochromosome s'accole au nucléole pour constituer un corps à forme de virgule, aplati contre la membrane nucléaire. Ces figures ont été reproduites dans mon travail antérieur (1927).

Quant aux autosomes, il est impossible de les distinguer les uns des autres. Cela tient à plusieurs causes, mais surtout à la petitesse des noyaux. Bien que le volume augmente régulièrement depuis la dernière télophase, il n'est pourtant pas assez grand pour permettre aux chromosomes de s'étaler. Ceux-ci sont relativement nombreux et longs; les filaments s'enchevêtrent de façon inextricable. Et même après le stade pachytène orienté, lorsque les filaments conjugués se répandent à travers le noyau, il n'est pas certain que les éléments isolés dans une coupe, même poursuivis sur une grande distance, correspondent à un chromosome complet. Les numérations ne sont d'aucun secours, puisqu'en raison de la longueur des chromosomes et le volume du noyau, il est rare que l'ensemble soit compris dans une seule coupe.

Les chromosomes ne deviennent observables qu'à un stade un peu plus avancé; ils se sont raccourcis et prennent tout d'abord la forme d'anneaux granuleux et irréguliers (fig. 18 et 19).

L'aspect de tétrade ne survient que plus tard et d'ailleurs n'affecte jamais chez *Gryllotalpa* une disposition en croix schématique, telle qu'elle apparaît chez d'autres espèces.

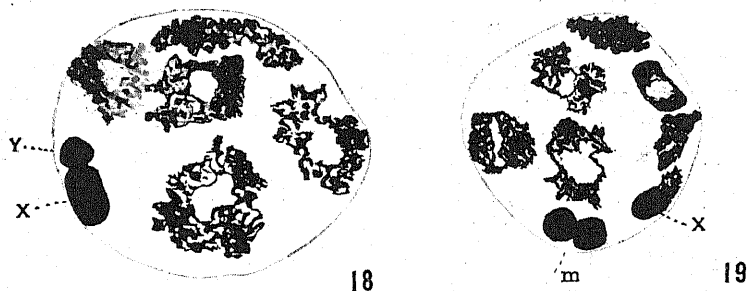


Fig. 18, 19. 18. Spermatocyte I. *Gryllotalpa gryll.* race septentrionale; à gauche, le couple X-Y.  $\times 3500$ . 19. id. race méridionale. A droite, le chromosome X; en bas, le microchromosome m.  $\times 3500$ .

A ce stade, la forme septentrionale diffère de la forme méridionale. La première possède six chromosomes bivalents: cinq anneaux et le couple X-Y. Il est rare que l'on puisse observer tous les anneaux de face; généralement appliqués contre la membrane nucléaire, ils se présentent tantôt de face, à plat, tantôt plus ou moins de profil. La fig. 18 est particulièrement heureuse, puisqu'un seul anneau est vu de profil. Les autres, situés à des niveaux différents, ont été projetés sur un seul plan dans le dessin. Le noyau est donc complet et offre les six chromosomes du cyte. I. Parmi ceux-ci, un des anneaux est plus volumineux que les autres, qui de leur côté vont en décroissant régulièrement. Le couple X-Y s'en distingue facilement par sa compacité et sa forme caractéristique.

La race méridionale compte huit chromosomes, dans le cyte I. Parmi ceux-ci, il y a six anneaux, en général plus petits que ceux de la race septentrionale. Mais considérés dans leur ensemble, on peut les décomposer en un élément de taille nettement plus volumineuse que les autres, ceux-ci étant régulièrement décroissants par rapport à celui-là. En plus, il existe un chromosome en biscuit ou deux globules accolés que l'on peut interpréter comme un anneau petit et contracté à l'extrême. Il existe d'ailleurs des transitions qui justifient cette manière de voir. Cet élément est évidemment le "microchromosome" de VOINOV, dont il est l'exacte réplique.

Quant au huitième chromosome, il est constitué par l'hétérochromosome X, accolé au nucléole. Ce dernier, avant de disparaître, se modifie et prend une structure granuleuse, rappelant vaguement celle des autosomes à ce stade. L'emploi de la triple coloration permet de constater que l'hétérochromosome se colore toujours comme la

chromatine, tandis que l'appendice, dérivé du nucléole, se colore comme ce dernier.

Le stade des chromosomes en anneaux est long. Il persiste jusqu'à la première mitose de maturation; le seul changement qui survienne encore est une contraction, une condensation progressive des chromosomes qui finissent par devenir des éléments allongés où une vague fente médiane rappelle l'orifice central des anneaux témoin de la constitution bivalente des chromosomes.

D. — *Métaphases I et II*. Les chromosomes sont allongés sur le fuseau; par conséquent, la vue de profil rend mieux compte de leur véritable forme qu'une vue polaire ou la coupe transversale. Dans la race septentrionale (fig. 4 à 6), la plaque est constituée de six éléments assez trapus, en groupe assez disparate, jamais en couronne régulière. Le couple X-Y n'est pas discernable, alors que de profil on le reconnaît aisément. (Cf. fig. 38 et 39, 1927).

Dans la race méridionale, la plaque équatoriale composée de huit chromosomes, affecte beaucoup moins d'ordre encore, mais elle possède ce détail caractéristique que le chromosome X est toujours en avance sur les autosomes. Ce qui se traduit sur les coupes transversales par la situation de l'X sur un autre plan que les autosomes, au-dessus ou en-dessous de ceux-ci par rapport à l'observateur (fig. 12 et 13; cf. avec les fig. 58 et 59 de 1927).

De plus, l'hétérochromosome est couché dans le plan de la coupe (insertion télomitique), tandis que les autosomes sont perpendiculaires à ce plan et intéressés par la section.

Malgré ces détails, les métaphases I des deux races présentent un indiscutable air de famille.

E. — *Chromosomes de Gryll. borealis*. — A titre de comparaison, les fig. 14 à 17 reproduisent des images de PAYNE (1916). On voit que les spermatogonies possèdent 23 chromosomes qui sont des bâtonnets, des crochets et des fers à cheval comme chez *Gryllotalpa gryll*, mais ils sont en général plus courts et plus épais. La métaphase I est composée de 12 éléments. Ici encore, les plaques équatoriales sont compactes, nullement ordonnées en couronnes.

Les remarques faites à propos de l'espèce européenne, s'appliquent donc également à l'espèce américaine. Les métaphases II comportent 12 ou 11 chromosomes selon la présence ou l'absence de l'hétérochromosome.

PAYNE donne peu de figures de la période d'accroissement, mais il semble qu'ici encore, on reconnaisse une série d'anneaux et un ou plusieurs microchromosomes du genre de celui signalé par VOINOV. Ce qui pourrait s'expliquer par la petitesse des chromosomes et leur nombre deux fois plus grand.



D'après tout ce qui précède, il résulte que le genre *Gryllotalpa gryllotalpa* L. possède en Europe une aire de dispersion assez étendue. Les spécimens, récoltés à différents endroits, ne se distinguent par aucun caractère extérieur. Mais par contre, on connaît trois races, différentes non seulement par le nombre des chromosomes, mais encore par les hétérochromosomes et la présence ou l'absence de chromosomes "différentiels" selon l'expression de MCCLUNG.

Nous nous trouvons donc, à première vue, en présence de faits diamétralement opposés à ceux de MCCLUNG. En effet, dans le genre *Mecostethus*, aussi limité que *Gryllotalpa*, des espèces distinctes par des caractères somatiques visibles, possèdent une garniture chromosomiale uniforme jusque dans des détails très spéciaux, au point que l'auteur croit pouvoir conclure à l'identité de tous les chromosomes de la garniture.

Faut-il en déduire que *Gryllotalpa* ne rentre pas dans la thèse énoncée par MCCLUNG, c'est à dire que, chez cette espèce, les chromosomes ne reflètent pas la constance des caractères somatiques externes? Je crois, pour ma part, que les différences ne sont pas aussi profondes qu'elles paraissent à première vue.

En effet, le point essentiel concerne le nombre de chromosomes, et il y a lieu en premier lieu de rechercher le pourquoi de cette divergence. J'avais, dans mon travail de 1927, émis l'hypothèse d'une fragmentation des chromosomes. L'identité des figures publiées par BARIGOZZI, a même permis à l'auteur de préciser quels chromosomes de la race septentrionale se seraient fragmentés pour constituer les chromosomes de la race méridionale: il s'agirait des couples deux et trois de la série. L'hétérochromosome Y, absent chez la race méridionale, se serait fusionné avec l'X chromosome dont les branches sont devenues légèrement inégales.

Je suis d'accord avec BARIGOZZI pour admettre la fragmentation. Mais en ce qui concerne le chromosome Y, je crois plutôt à une disparition qu'à une union avec l'X. Déjà WILSON a constaté que l'élément Y semble en régression et sa disparition complète a été observée chez plusieurs espèces. Mais BARIGOZZI en faisant appel au rapport nucléo-plasmatique, constate que le volume des noyaux chez les deux races méridionale et septentrionale sont égaux. BARIGOZZI en déduit que la quantité de chromatine est identique, tout en étant répartie sur un nombre plus grand de chromosomes.

La tendance à la fragmentation s'accroît dans la race roumaine. Elle aboutit ici au nombre de 17 chromosomes en moyenne, mais l'instabilité est telle que, chez une série d'individus, le nombre varie de 14 à 17. Nous ne savons pas si dans la race roumaine, le rapport nucléo-plasmatique est identique à celui des deux autres. A en juger



d'après les figures de VOINOV, en tenant compte du grossissement employé, il semble bien qu'il en soit ainsi.

Dans ces conditions, les chromosomes non fragmentés sont en tout cas, comparables; les autres le sont tout au moins quantitativement. Parmi les éléments identifiables, nous trouvons toujours un grand anneau, tranchant sur les autres qui vont en décroissant. Si nous faisons abstraction des hétérochromosomes qui sont reconnaissables précisément à cause de leurs allures aberrantes, un microchromosome apparaît en même temps que la fragmentation et se retrouve dans la race roumaine. Cette constatation est un argument en faveur de l'interprétation donnée plus haut: à savoir que le microchromosome serait un anneau de petite taille et très contracté.

Il existe d'ailleurs de nombreuses ressemblances: dans la forme des chromosomes, la disposition des plaques équatoriales, les détails de la période d'accroissement et les quelques éléments différentiels qui surviennent au cours de cette évolution. Mais ces ressemblances sont en partie masquées par la fragmentation des chromosomes. La cause profonde du phénomène qui est lié à l'habitat, nous échappe. Mais c'est la fragmentation qui altère la constance du nombre, et de la sorte trouble secondairement la similitude des autres caractères. Si l'on pouvait rétablir le nombre des chromosomes, il est probable que l'on effacerait du même coup les divergences secondaires.

Aussi je crois pouvoir conclure que *Gryllotalpa gryll.* ne constitue pas un exemple infirmant la règle établie par MCCLUNG. En tenant compte du mécanisme des variations numériques, les ressemblances apparaissent plus profondes et plus complètes qu'il ne semble à première vue. Par conséquent, ici encore, la constance somatique se double de celle de certains chromosomes, peut-être même, ainsi que le pense MCCLUNG, de tous les membres de la série.

#### Auteurs cités

- Barigozzi, C. Boll. Labor. Zool. Portici, 1933.—id. Zeit. f. Zellf. u. mikr. Anat. 18, 1933.  
McClung, C. E. Zeit. Zellf. u. mikr. Anat. 7, 1928.  
Ohmachi, F. Proc. imp. Acad. Tokio, 1923.  
Payne, J. Arch. Zellforsch. 9, 1912—ibid. 1912.  
Senna, A. Monit. zool. ital. 1911.  
Uvarov, B. P. Locusts and Grasshoppers. London, 1928.  
Voinov, D. Arch. de Zool. 54, 1914—63, 1925.  
Wilson, E. B. The Cell in Development and Heredity, 1925.  
Winiwarter, H. de. Arch. de Biol. 37, 1927.

## On the Special Autosomes with Reference to the Sex-determination of *Rumex acetosa*, L.

By

Y. Takenaka

Botanical Inst., Preparatory Dept. of Keijo Imperial University

### Introduction

Since the sex-chromosome was discovered in *Rumex acetosa* (Kihara and Ono, 1923a, b), *Elodea canadensis* (Santos, 1923), and *Melandrium* (Blackburn, 1923, Winge, 1923) the investigations of sex-chromosomes in angiosperms have been carried on very actively, and the latest compilation by Sinotô (1929) shows that 51 species of higher dioecious plants contain so-called sex-chromosomes. Nevertheless as far as the origin of sex-chromosome, or the phylogeny of the dioecious plants are concerned, there exists scarcely any explanation. The sex-chromosomes of *Rumex acetosa* have been, up to now, investigated by many observers. First Kihara and Ono (1923), and then Sinotô (1924) ascertained that the chromosome complements of *Rumex acetosa* were  $14 = 12a + 2X$  in the female and  $15 = 12a + X + 2Y$  in the male. Shimotomai and Ono (1928) discovered the triploid and tetraploid intersexual plants, and a little afterwards Ono (1928) the triploid female. It was also Ono (1930a, b) who published then his studies on many intersexual *acetosa* and discovered the male-intersexual plant carrying  $12a + X + 2Y$  chromosomes. Takenaka (1931) observed intersexual and male-intersexual plants containing  $12a + X + 2Y$  chromosomes and described new chromosomes as formed by translocation. Kihara and Yamamoto (1931) published their karyological investigations of abnormal forms of this species, while the latter successively reported the results of his karyological and physiological researches on the normal and abnormal plants (1932, '33, '34a, b, '35, and '36b). Ono (1935) concluded that the sex was determined by the ratio of the sex-chromosome (X) to the autosome. The writer (1936a) stated that the ratio of the number of the autosomes to that of the sex-chromosomes is generally in proportion to the degree of appearance of the sexual organs, and that exceptions to this were seldom found. Yamamoto (1936a) observed the new chromosome made by translocation. Satô and Sinotô (1935) discovered the triradial pairing and argued the occurrence of chiasmata of a tripartite sex-chromosome. Jensen (1936a, b) stated that the genus *Rumex* was compounded by hybridization and

that the so-called sex-chromosomes in *Rumex* were nothing more than an abnormality resulting from the previous hybridization of the species. It was explained by the writer (1935, '36b) that the sex-disturbing factors in the male- and female-intersexual plants carrying respectively  $12a + X + 2Y$  and  $12a + 2X$  were not dependent on X- and Y-chromosomes, but had some relation to a few autosomes. These plants were also produced by the writer through hybridization of normal wild plants collected from different districts. It was assumed by the writer from the results of both his karyological and his genetic studies, that the origin of the sex-chromosome in the dioecious plants which have not any remarkable phylogenetic relationship might be accounted for by aberrant chromosomes transformed owing to irregular nuclear divisions. The object of this paper is to give an account of some advanced researches on the sex-influencing chromosomes in the male- and female-intersexual plants.

### Material and Methods

The materials in the present experiments were the progeny of a male-intersexual plant, family number XI, having a diploid chromosome complement. The karyological studies on somatic cells of these offspring (Takenaka, 1936b) have shown that both male and male-intersexual plants received almost always  $12a + X + 2Y$  chromosomes, while both female and female-intersexual plants  $12a + 2X$ . For the research on the male- and female-intersexual chromosomes or factors, the following cross experiments were carried out:

1. Normal female  $\times$  male-intersex,
2. Female-intersex  $\times$  normal male,
3. Abnormal\* female  $\times$  male-intersex,
4. Female-intersex  $\times$  abnormal male,
5. Female-intersex  $\times$  male-intersex,
6. Abnormal female  $\times$  abnormal male,
7. Abnormal female  $\times$  normal male,
8. Normal female  $\times$  abnormal male.

Before maturation, some branches of the plants were enclosed in paraffin-paper bags and after a week or more pollination was carefully carried out by pollen-grains of the paternal plants which were kept in the laboratory room so as to avoid contamination with foreign pollens.

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\* The abnormal male and female plants are the offspring of family XI and of the female intersexes which are the progeny of family XI.

## Experiments

### Exp. I. Normal female $\times$ male-intersex

This breeding experiment was carried out to ascertain whether or not the male-intersexual chromosomes in the male-intersexual plants had any connection with the female-intersexuality. Pollination was tried in the springs of 1934 and 1935, and seeds were sown in the autumns of 1934 and 1935 respectively. Results were observed in the springs of 1935 and 1936.

One female-intersexual plant(?) grew up from the mating of "K-12  $\times$  171-17". In this plant, one flower only, among several hundreds, was observed to have an anther-like element. Whether it is to be taken

as female-intersex or female is uncertain. From these results it may be supposed that the male-intersexual plants are not concerned in the female-intersexuality. And two paternal autosomes ( $\alpha' + \beta'$ ) seem to be connected with the male-intersexuality when the certain of pollen-grains is neglected and the chromosome complements of paternal male-intersexes are all  $15 = \alpha\alpha' + \beta\beta' + 8$  autosomes + X + 2Y; because the ratio of  $\sigma$  to  $+\sigma$  is 38:12, i.e. about 3:1, and the pollen-grains for the male-intersexual  $F_1$  are a quarter of the whole. So the chromosome-formulae of the male-gametes are as follows:

$$\begin{aligned} &\alpha + \beta + 4 \text{ autosomes} + 2Y \\ &\alpha + \beta' + 4 \text{ autosomes} + 2Y \\ &\alpha' + \beta + 4 \text{ autosomes} + 2Y \\ &\alpha' + \beta' + 4 \text{ autosomes} + 2Y. \end{aligned}$$

### Exp. II. Female-intersex $\times$ normal male

This hybridization was examined to ascertain whether the female-intersexual chromosomes were concerned in the male-intersexuality or not. Pollination was tried in the spring of 1935, and seeds thus attained were sown in the autumn of the same year. The results were observed in the spring of 1936.

The results showed the facts that the maternal female-intersexual plants involved the male-intersexual chromosomes and that, in

Table 1.  $F_1$  results from normal female  $\times$  male-intersex

Maternal No.	Paternal No.	Classification of Progeny					
		$\sigma$	$+\sigma^*$	$\varphi$	$-\varphi^*$	$\varphi$	
148-6	121-14	5	7	—	—	—	27
K-12	171-17	—	—	—	—	1 (?)	8
K-12	17-134	21	1	—	—	—	43
K-10	17-134	3	1	—	—	—	8
K-5	17-134	2	3	—	—	—	24
K-22	17-144	5	—	—	—	—	13
K-26	17-147	2	—	—	—	—	3
Total		38	12	—	—	1 (?)	126

\*  $+\sigma$  and  $-\varphi$  indicate respectively male- and female-intersex.

Table 2.  $F_1$  results from female-intersex  $\times$  normal male

Maternal No.	Paternal No.	Classification of Progeny				
		$\sigma$	$+\sigma$	$\text{♀}$	$-\text{♀}$	$\text{♀}$
163-18	K-11	5	—	—	—	5
17-96	K-11	8	—	—	—	21
211-6	K-11	6	—	—	2	5
162-18	K-10	2	1	—	—	2
Total		21	1	—	2	33

the offspring, the female-intersexual individuals were much smaller in number than the female plants. From the latter fact the female intersex is supposed to be concerned with several autosomes. Accordingly all or most of the female plants (abnormal female) must

carry some of the female intersex-influencing chromosomes. As the number of plants resulting from the experiment was too small to permit determination of the number of the male- and female-intersex-influencing chromosomes carried from the maternal female-intersexual plants, the writer will give an account of it at some future time.

### Exp. III. Abnormal female $\times$ male-intersex

The results of Exp. II showed that the abnormal female generally involved some autosomes concerning the female-intersex and that the male-intersex depended on the several autosomes of the female-intersex. And also the results of Exp. I showed that the abnormal female was capable of containing some male-intersexual chromosomes. According to Exp. III, it may be possible to show several female-intersexual individuals, and the number of the male-intersexual plants may be greater than that in Exp. I.

Table 3.  $F_1$  results from abnormal female  $\times$  male-intersex

Maternal No.	Paternal No.	Classification of Progeny				
		$\sigma$	$+\sigma$	$\text{♀}$	$-\text{♀}$	$\text{♀}$
17-143	17-147	3	4	—	8	6
17-164	17-144	2	—	—	3	23
170-4	170-8	1	3	—	2	10
Total		6	7	—	13	39

$F_1$  results of Exp. III were in accord with the writer's expectation.

### Exp. IV. Female-intersex $\times$ abnormal male

It may be assumed that in the case of the abnormal male, which is the progeny of family XI, some of the female- and male-intersex-influencing chromosomes are involved among its chromosome-complement. Then many more female- and male-intersexual plants

Table 4.  $F_1$  results from female-intersex  $\times$  abnormal male

Maternal No.	Paternal No.	Classification of Progeny				
		$\sigma$	$+\sigma$	$\text{♀}$	$-\text{♀}$	$\text{♀}$
279-9	264-43	1	5	—	17	22
172-16	17-165	—	4	—	2	23
17-156	17-138	—	3	—	3	12
Total		1	12	—	22	57

should be found here than are found in Exp. II.

As shown in Table 4, many male- and female-intersexual plants were counted.

#### Exp. V. Female-intersex $\times$ male-intersex

In this experiment more female- and male-intersexual plants may generally be expected than in Exp. IV. The results were contrary to the writer's expectation, but the members of the male- and female-intersexual plants were found in greater number than in any other experiments except Exp. IV.

In this experiment, one intersexual plants was found. It might have germinated from the seed resulting from the combination of the abnormal gametes, one of which at least was made by incomplete meiosis, such as non-disjunction.

Table 5.  $F_1$  results from female-intersex  $\times$  male-intersex

Maternal No.	Paternal No.	Classification of Progeny				
		♂	+ ♂	♀	- ♀	♀
163-18	17-144	—	—	—	1	3
17-156	17-144	2	2	—	4	10
122-36	276-5	3	1	—	2	2
17-156	17-147	8	—	1	—	7
170-11	170-8	—	—	—	1	2
Total		13	3	1	8	24

Table 6.  $F_1$  results from abnormal female  $\times$  abnormal male

Maternal No.	Paternal No.	Classification of Progeny				
		♂	+ ♂	♀	- ♀	♀
17-160	17-165	1	—	—	—	15
17-143	17-138	5	2	—	6	32
Total		6	2	—	6	47

#### Exp. VI. Abnormal female $\times$ abnormal male

Results of this breeding experiment agreed with those expected, as shown by several male- and female-intersexual plants.

#### Exp. VII. Abnormal female $\times$ normal male

In this experiment, the writer anticipated the appearance of a few male-intersexual plants; and the result was satisfactory.

One intersexual plant found in this experiment might have germinated from the seed similar to that of the intersexual plant in Exp. V.

Table 7.  $F_1$  results from abnormal female  $\times$  normal male

Maternal No.	Paternal No.	Classification of Progeny				
		♂	+ ♂	♀	- ♀	♀
17-141	K-11	16	1	1	—	15
17-103	K-11	5	3	—	—	1
17-85	K-11	3	—	—	—	7
17-98	K-10	—	—	—	—	22
17-143	K-11	19	—	—	—	13
Total		43	4	1	—	58

#### Exp. VIII. Normal female $\times$ abnormal male

It was not necessary to carry out this experiment, because from the results of the preceding experiments no male- and female-inter-



Table 8.  $F_1$  results from normal female  $\times$  abnormal male

Maternal No.	Paternal No.	Classification of Progeny				
		$\sigma$	$+\sigma$	$\text{?}$	$-\text{?}$	$\text{?}$
K-24	17-138	5	—	—	—	21

sexual plants were expected to occur. This expectation was satisfactorily fulfilled, although the number of plants resulting from the experiment was too small.

### Discussion and Conclusion

In the writer's previous investigation (1936b), it was explained that the intersexuality of male- and female-intersexual plants, in which the chromosome-complements were diploid, was caused neither by X-chromosome nor Y-chromosome, but by particular autosomes.

In the present work, Exp. I showed that paternal male-intersexual plants gave male-intersexuality to some male offspring. In the results of this experiment, the male-intersexuality was seen as determined by two autosomes; for the ratio of male to male-intersex was about 3:1: but these phenomena are not so simple, because it is doubtful whether the paternal materials have all the same chromosome-complements and whether they contain anything in addition to the chromosome-members necessary for the male-intersexuality.

In Exp. II, it was pointed out that the female-intersexual plants gave female-intersexuality to some of their female offspring as was seen in the case of the male-intersexual offspring in the male-intersex. And now it is noteworthy that the male-intersexual chromosomes are included in the female-intersexual plants.

The results obtained by Exps. I and II were still more clearly confirmed by Exps. III, IV, V, VI, and VII, and it was found that both abnormal males and abnormal females carried some of the male- and female-intersexual chromosomes.

As suggested in the writer's previous papers (1931, '36b), these male- and female-intersexual chromosomes are supposed to be transformed from normal chromosomes by irregular divisions, such as deficiency, inversion, translocation, interchange, and attachment. Yamamoto (1935) also has described a case of simple translocation of a certain autosome of *Rumex acetosa*. Jensen (1936b) stated that sex-chromosomes in *Rumex* were nothing more than abnormality resulting from the previous hybridization of the species. Nevertheless, Ono (1935) and the writer (1936b) came to a definite conclusion in the studies of *Rumex acetosa* that an X-chromosome involves a female-factor or gene and autosomes have connection with a male-factor or gene. As pointed out by Jensen (1936a), some of the general phenomena seen in hybrids were also observed karyologically by the writer (1931, 1936b).



Accordingly it is suggested that various forms of *Rumex acetosa* exist and are co-mixed in the fields. Winge (1932) stated that sex-chromosomes could be experimentally changed into autosomes. Marsden-Jones and Turrill (1935) observed six sexual types of *Ranunculus acris*, and stated that these variations were not due to the differences in the number of chromosomes. Hirata (1927) investigated female- and male-intersexual plants of hemp and stated that dioecious forms had been evolved from hermaphrodite ones. Many investigators have studied the sex of maize. For example, Jones (1934) announced that the chromosomes of normal maize always included many male- and female-genes, and that in the dioecious plants specific genes promoted one sex and suppressed the other. Akemine (1935) made a study of various sex expressions of *Coptis japonica* and observed no heteromorphic pair in them. Von Ubisch (1936) tried to breed the hermaphroditic plants in *Antennaria dioica*, although no karyological investigation was carried out, and found a certain relationship among male, intersex and female.

From what has been mentioned about *Rumex acetosa*, the writer has come to the conclusion that some of the autosomes of the male- and female-intersexual plants carried respectively the male- and female-genes; that the male-intersexual chromosomes weaken the function of autosomes (maleness) and also the female-intersexual chromosomes suppress the function of X-chromosomes (femaleness); and that the female-intersexual factors or genes are concerned with more numerous chromosomes than the male-intersexual factors or genes are. The origin of these sex-influencing chromosomes seems to be due to various irregular divisions of some autosomes. From this point of view, reversible phenomena are assumed between all hermaphroditic and dioecious plants in the vegetable kingdom, through irregular chromosome divisions or abnormal gene distributions.

#### Literature Cited

- Akemine, T. 1935. On the sex expression of *Coptis japonica*, Makino. Jour. Facul. Sci. Hokkaido Imp. Univ. V, 5, 1-7.
- Blackburn, K. B. 1923. Sex chromosomes in plants. Nature 112, 687-688.
- Hirata, K. 1927. Sex determination in hemp. Jour. Genet. 19, 65-79.
- Jensen, H. W. 1936a. Meiosis in *Rumex*. I. Polyploidy and the origin of new species. Cytologia 7, 1-22.
- 1936b. Meiosis in *Rumex*. II. The origin and behaviour of the so-called sex-chromosomes in *Rumex*. Ib. 7, 23-34.
- Jones, D. F. 1934. Unisexual maize plants and their bearing on sex differentiation in other plants and animals. Genetics 19, 552-567.
- Kihara, H. and Ono, T. 1923a. Cytological studies on *Rumex*. I. Chromosomes of *Rumex acetosa*, L. Bot. Mag. (Tokyo) 37, 84-90.
- and — 1923b. Cytological studies on *Rumex*. II. On the relation of chromosome number and sexes in *Rumex acetosa*, L. Ib. 37, 147-149.

- Kihara, H. und Yamamoto, Y. 1931. Karyomorphologische Untersuchungen an *Rumex acetosa*, und *Rumex montanus*, Desf. Cytologia 3: 48-118.
- Marsden-Jones, E. M. and Turrill, W. B. 1935. Studies in *Ranunculus*. III. Further experiments concerning sex in *Ranunculus acris*. Jour. Gent. 31, 363-378.
- Ono, T. 1928. Further investigations on the cytology of *Rumex*. I-V. Bot. Mag. (Tokyo) 42, 524-532.
- 1930a. Further investigations on the cytology of *Rumex*. IV-VIII. Ib. 44, 168-176.
- 1930b. Chromosomenmorphologie von *Rumex acetosa*. Sci. Rep. Tohoku Imp. Univ. IV. 5, 415-422.
- 1935. Chromosomen und Sexualität von *Rumex acetosa*. Ib. V. 10, 41-210.
- Santos, J. 1923. Differentiation among chromosomes in *Elodea*. Bot. Gaz. 75, 42-59.
- Satô, D. and Sinotô, Y. 1935. Chiasma studies in plants. III. Chromosome pairing and chiasma behaviour in the male *Rumex acetosa* with special reference to the tripartite sex-chromosome. Jap. Jour. Genet. 11, 219-226.
- Shimotomai, N. and Ono, T. 1923. Triploid and tetraploid intersex of *Rumex acetosa*, L. Bot. Mag. (Tokyo) 42, 266-270.
- Sinotô, Y. 1924. On chromosome behaviour and sex determination in *Rumex acetosa*, L. Bot. Mag., (Tokyo) 43, 153-162.
- 1929. Chromosome studies in some dioecious plants, with special reference to the allosomes. Cytologia 1, 109-191.
- Takenaka, Y. 1931. Further reports of cytological and genetic investigations of *Rumex acetosa*, L. I. New chromosome and chromosome-fragments. Bot. Mag. (Tokyo) 45, 475-486.
- 1935. A new remark on the sex of *Rumex acetosa*, L. Bot. & Zool. (Tokyo) 3, 2178-2179.
- 1936a. Further reports of cytological and genetic investigations of *Rumex acetosa*, L. II. Polyploid plants and those offsprings. Ib. 4, 1193-1204.
- 1936b. Further reports of cytological and genetic investigations of *Rumex acetosa*, L. III. On male- and female intersex plants. Jour. Chosen Nat. Hist. Soc. 21, 58-76.
- Von Ubisch, G. 1936. Genetic studies on the nature of hermaphroditic plants in *Antennaria dioica* (L). Gärtn. Genetics 21, 282-294.
- Winge, Ö. 1923. On sex chromosomes, sex determination and preponderance of females in some dioecious plants. C. R. Trav. Lab. Carlsberg 15, 1-25.
- 1932. The nature of sex chromosomes. Proceed. Sixth International. Cong. Genetics 1, 343-355.
- Yamamoto, Y. 1932. A cyto-genetical study on *Rumex acetosa* with 3 Y-chromosomes. Kwagaku (Science) 2, 5-6.
- 1933. An autohexaploid plant of *Rumex acetosa*. Jap. Jour. Genet. 8, 2-3.
- 1934a. Karyogenetische Untersuchungen bei der Gattung *Rumex* I. Hetero- und Euploidie bei *Rumex acetosa*, L. Cytologia 5, 317-336.
- 1934b. Reifungsteilungen bei einer asynaptischen Pflanze von *Rumex acetosa*, L. Bot. & Zool. (Tokyo) 2, 1160-1168.
- 1935. Karyogenetische Untersuchungen bei der Gattung *Rumex*. II-III. Jap. Jour. Genet. 11, 6-17.
- 1936a. Karyogenetische Untersuchungen bei der Gattung *Rumex*. IV. Ein Fall von einfacher Translocation bei *Rumex acetosa* L. Cytologia 6, 407-412.
- 1936b. Über den Einfluß von Kaliumchlorat auf getrenntgeschlechtige Pflanzen. Jap. Jour. Genet. 12, 73-80.

## Wirkliche, abgeleitete und fragliche Weizenroggenbastarde (Triticale-Formen)

Von

Professor Dr. Erich Tschermak-Seysenegg, Wien

### 1. Beobachtungen am RIMPAU'schen WR-Bastard

In der Zeitschrift „Der Züchter“ 7. Jahrgang 1935, Seite 228–233 berichten M. LINDSCHAU und E. OEHLER über den von W. RIMPAU (Schlanstedt) im Jahre 1888 erzeugten Weizenroggenbastard (WRB), der sich erst jetzt als ein konstant intermediärer additiver Bastard mit der somatischen Chromosomenzahl  $n = 56$  ( $= 2 \times 14 + 2 \times 14$ ) erwiesen hat. Amsrath Dr. h. c. WILHELM RIMPAU<sup>1)</sup> hat seinerzeit von der  $F_2$  und den nächsten Generationen dieses konstanten Bastardes einige Ähren oder Körner an Freunde und Bekannte abgegeben, wie wir heute wissen auch an das landw. Institut in

1) Wenn heute daran erinnert wird, daß bereits JOHN GOSS und LAXTON (vergl. H. E. ROBERTS *Plantshybridization before MENDEL*. Princeton University Press. 1929. S. 102–105) bei Kreuzungen grünkörniger Erbsen mit gelbkörnigen die Dominanz von Gelb über Grün erkannt und in der 2. Samengeneration die Konstanz der herausgespaltenen grünen und eines Teiles der gelben beobachtet und auf diese Weise wenigstens den Tatbestand eines MENDEL'schen Falles nachgewiesen haben, so muß andererseits darauf aufmerksam gemacht werden, daß W. RIMPAU (Kreuzungsprodukte landwirtschaftlicher Kulturpflanzen. Berlin. Verl. P. Parey 1891 Sonderabdruck aus: Landw. Jahrbücher 1891.) bei seinen zahlreichen Bastardierungen von Getreiderassen die Uniformität der ersten Bastardgenerationen sowie das Konstantwerden mancher Merkmale bereits in der dritten Generation, z.B. der Begrannung bei begrannnten  $\times$  unbegrannnten Formen von Weizen und Gerste sowie der Vierzeiligkeit bei Kreuzungen von zweizeiligen mit 4 zeiligen Formen, klar erkannt hat (S. 31). Er sagt ferner auf Seite 24 „Nach meinen Beobachtungen an dieser Kreuzung Steudel Gerste: zweizeilig, bespelzt, begrannt, schwarz  $\times$  Gabelgerste: vierzeilig, nackt, Kapuze, weiß, vermute ich, daß kein physiologisches Hindernis vorliegt, jede beliebige Kombination von zwei vorhandenen Gerstenformen (soll heißen: Formeigenschaften) durch Kreuzung zu erzielen“. (Von mir gesperrt!) Aus seinen genauen Aufzeichnungen konnte ich seinerzeit bereits im Jahr 1901! das wahrscheinliche Verhalten einer ganzen Reihe von morphologischen Merkmalen bei unseren Getreidearten (Weizen, Gerste und Hafer) herauslesen und darüber berichten. (Zeitschrift für das landw. Versuchswesen in Oesterreich 1901. Über Züchtung neuer Getreiderassen mittels künstlicher Kreuzung. Kritisch-historische Betrachtungen). RIMPAU war auch sehr erfreut, daß ich ihm schrieb, daß er bei zahlenmäßiger Feststellung des Verhaltens der von ihm studierten Merkmale in mehreren Generationen sehr nahe daran gewesen wäre, die MENDEL'schen Gesetze wieder zu entdecken. Er zitiert auch FOCKES Buch „Pflanzenmischlinge 1881“ bei Beschreibung seiner Erbsenkreuzungen und ganze Stellen aus demselben, so daß es direkt Wunder nimmt, daß er nicht auf MENDEL'S Citat bei FOCKE gestossen ist. Jedenfalls haben wir allen Grund dem Altmeister der Deutschen Getreidezüchtung für seine mustergiltigen Beobachtungen und Aufzeichnungen dauernd dankbar zu sein.

Halle (wahrscheinlich direkt an JULIUS KÜHN), wie HOLDEFLEISS<sup>1)</sup> berichtet, sowie auch an A. v. LIEBENBERG in Wien. Hier fand ich diesen Bastard schon im Jahre 1899 im Garten der Hochschule für Bodenkultur angebaut vor. Wir besitzen hier den Bastard sowohl in „begrannter“ als in „unbegrannter“ Form. Nach den Aussagen des Gärtners der Lehrkanzel für Pflanzenproduktionslehre soll die begrannte Form schon im Jahre 1899 hier angebaut gewesen sein, während die grannenlose Form erst später, vielleicht sogar durch mich selbst nach Wien gebracht worden sei. Ich besitze selbst in meiner Sammlung drei Ähren des RIMPAU'schen WRB in unbegrannter Form, die ich entweder vor ca 35 Jahren von RIMPAU selbst oder von Hofrat LIEBENBERG erhalten habe. RIMPAU war nämlich so liebenswürdig mir für einen Vortrag, den ich im Jahre 1902 im Klub der Land- und Forstwirte in Wien gehalten habe, seine Getreide-Kreuzungsprodukte, auf Tafeln aufgeklebt unter Glas und Rahmen, zur Verfügung zu stellen und es kann sein, daß er mir damals auch das Muster seines WRB geschenkt hat, das ich, wie gesagt, heute noch besitze. Zwei Ähren sind noch vollständig erhalten, von der dritten, die abgebrochen, nur mehr etwa ein Drittel. Die Spindel-längen der beiden unverletzten Ähren betragen 13.2 resp. 13.5 cm mit je 22 resp. 23 Ährchen. Die eine von den unverletzten sowie die abgebrochene Ähre sind fruchtbar, die andere unverletzte ist vollständig steril. Die fruchtbare unverletzte Ähre, deren Körner ich ihr erst jetzt entnommen habe—nicht ein einziges Korn war ausgefallen!—, enthielt 19 Korn. Die meisten Ährchen entwickelten ein Korn, einzelne waren auch vollständig steril, andere enthielten auch zwei, jedoch niemals drei Körner. Die Ährenfarbe ist hellbraun, die Ähre grannenlos nur die Deckspelzen der Ährchen an der Ährenspitze tragen ganz kurze bis zu 1 cm lange Grannenstummeln. Die Ähre ist etwas breiter, als die von RIMPAU<sup>2)</sup> auf Tafel 6 Abb. 58 abgebildete Ähre, hingegen annähernd so breit, wie die von F<sub>2</sub> abgebildete Ähre Fig. 59, die bedeutend länger war und ca 31 Ährchen trug. Die F<sub>1</sub>-Pflanze des RIMPAU'schen Bastardes produzierte nur 15 Korn, die Ähren waren viel schmaler und trugen längere Grannenstummeln an der Ährenspitze. Es ist eine mir wohl bekannte Erscheinung, daß die unfruchtbaren oder stark sterilen grannenlosen Weizenroggenabkömmlinge längere Grannenansätze zeigen, als die fruchtbaren; auch sind die fruchtbaren Ähren infolge stärkeren Abspreizens der Spelzen natürlich breiter als die sterilen. Die fruchtbarste Ähre des RIMPAU'schen WRB enthielt, wie RIMPAU mitteilte, 53 Korn und dürfte nach der Abbildung zu schließen ca 31 Ährchen, demnach

1) Vergl. M. LINDSCHAU u. OEHLER, S. 229.

2) Vergl. W. RIMPAU Kreuzungsprodukte l.c.

1.71 Korn pro Ährchen entwickelt haben. Es ist allerdings anzunehmen, daß RIMPAU nicht die längsten und fruchtbarsten Ähren abgegeben hat. Die ganz sterile Ähre stammt vermutlich auch von einer ganz sterilen Pflanze der  $F_2$  von RIMPAUS Bastarden, von welchen er 12 Pflanzen erzogen hatte; denn der spätere Nachbau, also ab  $F_3$ , dürfte nach unserem Material zu schließen, wenn auch etwas sterile, so doch nicht mehr völlig unfruchtbare Pflanzen entwickelt haben. Auch bei meinen *Aegilotriticum*- und *Haynaldtricum*-Bastarden nahm die Fruchtbarkeit in  $F_2$  deutlich zu, weshalb auch die Ähren breiter und ansehnlicher wurden; ja, sie erfuhr in  $F_3$  noch eine weitere Steigerung, um von da ab ziemlich gleich zu bleiben. Der Nachbau der begannten und unbegannten RIMPAU'schen WRB stimmt genau mit dem Ährentypus der von RIMPAU abgebildeten, ebenso mit den Figuren bei LINDSCHAU und OEHLER überein; die begannte Form unterscheidet sich nur durch den Besitz der Grannen von der unbegannten. Ich habe von den beiden Formen je eine besonders gut entwickelte Ähre gemessen und ihre Fruchtbarkeit bestimmt (Vergl. Abb. 1). Die begannte Ähre hat eine Spindelänge von 16 cm, trägt 28 Ährchen, die 3–4 blütig sind und ab und zu 3 Körner entwickeln; die Ähre enthielt 55 Korn, also ca 2 Korn pro Ährchen. Die unbegannte Ähre hat eine Ährenspindel von 15.8 cm Länge mit 28 Ährchen, die des öfteren 3 Körner entwickelten. Die Ähre enthielt 63 Korn also 2.33 Korn pro Ährchen.<sup>1)</sup> Die Fertilität stimmt demnach mit dem Hallenser Material, bei



Abb. 1. Begannte und unbegannte Form des RIMPAU'schen Original Weizenroggenbastardes.

1) Weit geringeren Ertrag erhielten LINDSCHAU u. OEHLER (0,5) sowie KATTERMANN ( $1,03 \pm 0,038 - 1936$ ).



welchem 2 Korn pro Ährchen angegeben werden, gut überein und hat später nicht mehr zugenommen. Beide Bastardformen meines Nachbaues haben die somatische Chromosomenzahl  $2n = 56$ . Nach meiner Theorie der Chromosomenaddition (1) entspricht diese Zahl nicht irgendwelcher Form von echter Polyploidie, sondern einer Kernchimäre d.h. dem Nebeneinandervorkommen eines diploiden Satzes

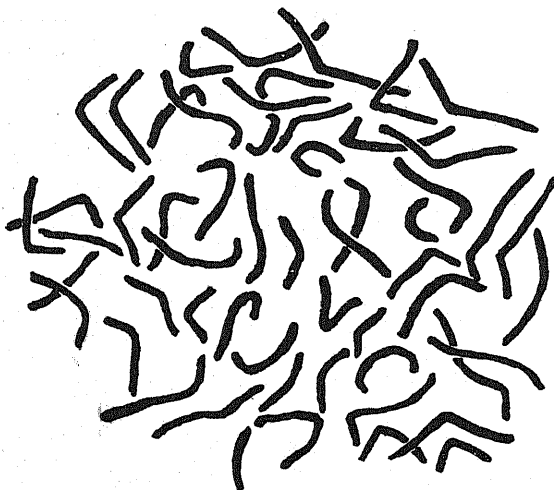


Abb. 2. Begrannter Weizenroggenbastard. Wurzelspitze, Metaphase,  $2n = 56$ . Vergr. ca 3400.

von Weizenchromosomen (*Tr. vulgare* 21/42) und eines diploiden Satzes von Roggenchromosomen (*Sec. cereale* 7/14), also der Formel  $(2 \times 21) + (2 \times 7) = 56$  für somatische Mitosen ab  $F_2$ , somit eine Amphidiploidie. Die cytologische Analyse<sup>1)</sup> (vergl. Abb. 2), welche die somatische Metaphase mit  $2 \times (21 + 7) = 56$  Kernschleifen in  $3400 \times$  Vergrößerung darstellt, verdanke ich Frl. R.

WUNDERLICH, die beim Chromosomenzählen in dankenswerter Weise von ihrem Lehrer Prof. Dr. K. SCHNARF unterstützt wurde.

Wie kann nun die begrannnte Form des RIMPAU'schen WRB entstanden sein, die wie gesagt, schon vor dem Jahre 1899 aufgetreten sein mußte? Die Annahme, daß es sich um eine spontane Abänderung des ursprünglich grannenlosen WRB von RIMPAU schon in den ersten Jahren seines Nachbaues handelt, gewinnt an Wahrscheinlichkeit, wenn wir die Beobachtungen berücksichtigen, die RIMPAU selbst an der Mutter seines WRB, dem „grannenlosen“ braunährigen Sächsischen Landweizen mit ca 22 Ährchen pro Ährenspindel gemacht hatte. Der Vater war der langbegrannnte, langährige Schlanstedter Roggen mit ca 40 Ährchen an der Spindel. Der Bastard war natürlich grannenlos und sollte, da er sich als konstanter Additionsbastard erwies auch bezüglich der Grannenlosigkeit konstant bleiben. Spaltet ja doch mein *Aegilotriticum* II = *Aegilops ovata* (gelbkörnig)  $\times$  *Triticum durum* Arraseita (blaukörnig) auch nicht bezüglich der Samen-

1) Die cytologischen Arbeiten an meiner Lehrkanzel erfreuen sich der Förderung durch die deutsche Forschungsgemeinschaft (Notgemeinschaft der Deutschen Wissenschaft). Dieser sei auch an dieser Stelle der verbindlichste Dank ausgesprochen.

schalenfarbe, die vielmehr dauernd blau bleibt. Nun berichtet aber RIMPAU (S. 8), daß der sächsische rote grannenlose Landweizen die Neigung hat ab und zu begrannte, sonst genau mit der Ausgangspflanze übereinstimmende Pflanzen zu entwickeln, eine Erscheinung, die er als spontane Variation bezeichnete und auch bei anderen Kolbenweizen-Sorten beobachtete. Ich selbst habe solche spontane Variationen—besser gesagt: Mutationen, da die einmal aufgetretene Veränderung konstant bleibt—bei dem fast unbegrannten „RIMPAU'schen Bastardweizen“ des öfteren beobachten können. Die in großen Beständen wiederholt auftretenden begrannten, vollständig konstant bleibenden Formen stimmen im Ährentypus völlig mit der grannenlosen Form überein. In diesem Falle sind übrigens, wie RIMPAU berichtet, beide Eltern grannenlos, nur zeigen sowohl die Mutter, der frühe rote Amerikanische Weizen, sowie der väterliche Squarehead im obersten Ährendrittel ab und zu kurze Grannenstummeln. Auch der Original-Bastard von RIMPAU Fig. 48 auf Tafel 5 zeigt solche und noch stärker der Nachbau (vergl. die Abbildung der Ähre in dem Buche „Deutsche Hochzuchten“ herausgegeben von Dr. RAMM Verl. P. PAREY Berlin 1926. Seite 90, wo übrigens fälschlich der amerikanische Landweizen als „begrannt“ angegeben wird). Es liegt also nahe, daß auch der grannenlose RIMPAU'sche WRB die Neigung hatte oder vielleicht auch heute noch hat spontan nach der Begrannung hin zu mutieren.<sup>1)</sup> Hier sei nur kurz die Möglichkeit angedeutet, daß es sich, speziell bei nicht—spaltenden Artbastarden, um eine gelegentliche Auslösung einer Mutation durch Bastardierung—u. zw. bei Gegebensein einer „mutationsbereiten“ Elternform—handelt. Der Nachbau des RIMPAU'schen WRB hat heute noch eine verhältnismäßig lange ansehnliche Ähre und blüht mit weitgeöffneten Spelzen, was natürlich bei der begrannten Form infolge des weiten Abspreizens der Grannen noch deutlicher zum Ausdruck kommt, und wird, wenn zu dieser Zeit noch ein Roggen blüht (Infektionsquette!), stark vom Mutterkorn befallen. Bei beiden Formen ist der Halm unterhalb der Ähre nicht behaart. Die Kornproduktion dieser spätblühenden und spätreifenden Bastarde wäre an sich sehr befriedigend, da doch jedes Ährchen der langen Ähren mindestens zwei Körner entwickelt. Doch ist stets nur ein Teil der großen Körner normal entwickelt, die anderen sind eingeschrumpft und keimen schlecht. Das war auch der Grund, warum der RIMPAU'sche WRB niemals in größerem Maßstabe vermehrt wurde, obwohl, wie ich weiß, solche Versuche begonnen worden sind. Unterdessen sind auch von anderer

1) Nach einer brieflichen Mitteilung des Enkels von W. RIMPAU, Herrn J. W. Rimpau, finden sich bereits in der vom ersteren angelegten Schausammlung der WR Bastarde einzelne begrannte Individuen.



Seite (MEISTER, TAYLOR and QUISENBERRY und MÜNTZING<sup>1)</sup>) solche konstante WRB unter Chromosomenaddition, also mit 28/56 gewonnen worden, die sich wohl alle für die erhoffte Steigerung der Körnerproduktion nicht eignen werden. KATTERMANN setzt gewisse Hoffnungen auf eine neuerliche Kreuzung von Additionsbastarden rassenverschiedener Weizen- und Roggenformen.

## 2. Über abgeleitete WR-Bastarde

Auch von den Rückkreuzungsprodukten der von einer sterilen  $F_1$  abstammenden WRB mit Weizen oder Roggen scheinen diejenigen Typen, die der  $F_1$  in ihrem Äußeren nahe kommen, aber selbst diejenigen mit Weizen-Äußerem, aber mit deutlich behaarten, kurzen Halmen<sup>2)</sup> unterhalb der Ähre, bezüglich ihrer Fruchtbarkeit und Ausbildung ihrer Körner zu enttäuschen. Auch ist es mir—im Gegensatz zu KATTERMANN<sup>3)</sup>—bisher nicht gelungen die Behaarung unterhalb der Ähre, die auch äußerlich einen, wenn auch abgeleiteten WRB kenntlich machen würde, erblich festzuhalten. Individuen aus scheinbar bereits konstanten Linien (68 Indiv. in  $F_{11}$ ) spalteten in  $F_{12}$  bezüglich der Halmbehaarung doch wieder auf, ja sie erzeugten in manchen Fällen wieder nur Individuen mit fehlender Halmbehaarung. Allerdings waren die Individuen gegen Fremdbestäubung nicht geschützt, die aber kaum in Frage kommt, da der Ährentypus konstant blieb. Eine praktische Verwertung scheinen wenigstens bis heute nur solche Abkömmlinge aus WRB zu versprechen, die durch Rückbastardierung einer unfruchtbaren  $F_1$  aus Kombinationen von *Triticum vulgare*—mit *Secale cereale*—Formen, rückgekreuzt mit *Triticum vulgare*, erhalten worden sind. Unter solchen, dem *Triticum vulgare* völlig gleichenden Individuen, die auch cytologisch mit 21/42 als *Triticum vulgare* gleichend bestimmt worden sind, lassen sich durch fortgesetzte Individualauslese vollständig fruchtbare Formen gewinnen, die bezüglich Ertrag aber auch bezüglich der Backfähigkeit des Mehles recht befriedigen. Sowohl in Rußland, Deutschland (Salzmünde), in Österreich und in der Tschechoslovakei sind Anbauversuche mit solchen Formen auch auf leichten sandigen, sogenannten Roggenböden im Gange, um die Eignung der WRB als Roggenersatz gerade auf solchen Böden zu prüfen. Sowohl begrannnte als unbegrannnte Formen solcher Herkunft sind von dem Nachbau meiner eigenen WRB in Züchtung. Leider sind in diesem Jahre einzelne

1) Vergl. die Literatur bei ARNE MÜNTZING (Svalöf) Der Züchter 1936 S. 188–191 Über die Entstehungsweise 56 chromosomiger Weizen-Roggen-Bastarde.

2) Daß mit dem Behaarungsmerkmal gleichzeitig auch ein halmverkürzender Faktor, wie KATTERMANN beobachtet hat, auftritt, kann ich bestätigen.

3) G. KATTERMANN Pflanzenbau Heft Juli 1936. Genetische Ergebnisse bei WRB bis  $F_4$ . Mitteilung II. Über anscheinend konstante Bastardformen mit behaartem Halm, ihre Abstammung und ihre Merkmalsverhältnisse.

dieser Linien, speziell der unbegrannten Formen, stark vom Gelbrost befallen worden, wodurch der Ertrag sehr gedrückt wurde. Es ist selbstverständlich, daß nun neuerdings der fruchtbare, für praktische Zwecke aber bisher nicht geeignete WRB mit 56 Chromosomen mit verschiedenen Weizenformen (*Tr. monococcum* 7/14, *Tr. dicoccum* und *diccoides* 14/28, *Tr. durum* 14/28, *Tr. turgidum* 14/28, *Tr. vulgare* 21/42) und Roggenformen (durchwegs 7/14) rückbastardiert wird, in der Erwartung vielleicht doch Gigas-Formen—unter reichlicher Chromosomenaddition—zu erhalten, die in ihren die Länge der bisher gezüchteten *Triticum vulgare*-Formen übertreffenden Ähren auch voll ausgebildete Körner entwickeln und auf diese Weise bisher noch nicht erreichte Erträge liefern könnten. Meine diesbezüglich schon vor einer Reihe von Jahren—als ich noch nicht wußte, daß der WRB von RIMPAU ein konstanter Additionsbastard ist—eingeleiteten Versuche ergaben allerdings bisher kein praktisches Resultat.

### 3. Fragliche WR-Bastarde

Schließlich will ich als fraglichen WRB noch den von KATTERMANN (2) seit dem Jahre 1928 bearbeiteten „blaukörnigen“ Weizen erwähnen. Derselbe soll aus einer Kreuzung einer Speltoidform des Moosbrunner begrannten Dickkopfweizens *Triticum vulgare* var. *erythrospermum* (21/42) × *Aegilops ovata typica* (14/28) entstanden sein. Schon in F<sub>1</sub> befand sich bei Beschreibung einer Pflanze (Nr. 5) die Bemerkung über eine blaugrüne Färbung von Körnern. Die blaugrüne Färbung der Aleuronschicht ist es, die den Weizen „roggenfarbig“ erscheinen läßt. Die in den ersten Generationen festgestellte z.T. weitgehende Sterilität sowie das Auftreten verschiedener Anomalien an den Körnern und Ähren sowie cytologische Untersuchungen bewogen KATTERMANN zu der Annahme, daß es sich um einen Gattungsbastard, wie er in den Akten beschrieben war, handle. Neu ausgeführte *Speltoid-Aegilops* Kreuzungen liessen aber bei KATTERMANN Zweifel über die Beteiligung von *Aegilops ovata* an dem Ergebnis der ursprünglichen Kreuzung aufkommen und er ist jetzt der Ansicht, daß wir es möglicherweise mit Kreuzungsprodukten zu tun haben, welche aus einer ungewollten, freien Bastardierung mit Roggen hervorgegangen sind. Dr. KATTERMANN war so liebenswürdig mir von seinem Material eine Probe abzugeben, die ich zu neuen Kreuzungen mit früher reifenden Weizen verwendete. Das ursprüngliche Material lieferte nämlich einen spätreifen, wenig ertragreichen blaukörnigen Weizen mit mehr länglichen Körnern. Durch Einkreuzung mit früherreifenden *Triticum vulgare*-Formen habe ich jetzt blaukörnige, früher reifende, vollständig fertile Weizen mit mehr rundlichem Korn erhalten, die eine überraschend gute Kleber-

qualität des Mehles aufweisen. Die Erträge dieser neuen blau-körnigen Weizen lassen aber noch immer viel zu wünschen übrig. Doch besteht kein Zweifel, daß die Blaukörnigkeit auch in ertragreichere Formen hineinkombiniert werden kann. Erst dann wird eine Entscheidung gefällt werden können, ob die immerhin bestechende Blaukörnigkeit in Kombination mit guter Backfähigkeit des nur wenig dunkleren Mehles für die Praxis eine Bedeutung haben könnte. Es ist nun interessant, daß wir korrespondierend zu unseren Kulturgetreideformen bei einzelnen Wildformen oder wenigstens den Wildformen noch nahestehenden Getreidearten eine blaugrüne Färbung der Aleuronschicht und daher auch Xenien an den Fruchtständen feststellen können. So ist dies bei einzelnen Wildformen des Roggens sowie bei *Triticum aegilopoides*-Formen, aber, wie ich glaube, auch bei *Aegilops*-Formen z.B. bei *Aegilops sharonensis* und anderen *Aegilops*-Formen, ferner bei *Agropyrum*-Formen, z.B. bei *Agropyrum giganteum*, sowie auch bei *Hordeum bulbosum* der Fall. In dem Garten der Hochschule für Bodenkultur in Wien sind auf einzelnen Rasenflächen *Hordeum bulbosum*-Pflanzen aufgegangen, die gewiß von meinem in Töpfen gezogenen *Hordeum bulbosum*-Material abstammen, das ich seinerzeit von Prof. KOERNICKE sen. aus Bonn erhalten hatte. Bei genauerer Untersuchung der Samen und der brüchigen Ähren im vorigen Jahre konnte ich feststellen, daß in den einzelnen Ähren einiger Pflanzen gelbe und intensiv dunkelgrün sowie lichtviolett gefärbte Körner vorkommen, die grünen oder violetten stets in der Minderzahl. Es würde dies darauf hindeuten, daß auch hier, wie beim blauen Weizen, der Erbgang der Aleuronfärbung ein rezessiver ist. Da ich bereits homozygot-grünkörnige *H. bulbosum*-Pflanzen herangezogen habe, werde ich schon im nächsten Jahre bereits über die Xenien bei *Hordeum bulbosum* Genaueres berichten können. KATTERMANN nimmt einen Erbfaktor F für blaukörnigen Weizen (FFhh) und einen Hemmungsfaktor H bei gewöhnlichen Weizen (ffHH) an. Die Klassifikationsschwierigkeiten bei der Selektion sind erhebliche, da die Färbung manches Mal nur in Spuren auftritt. Auch kommen des öfteren in rein blauen Stämmen einzelne gelbe Körner vor, die meines Erachtens nicht auf Fremdbestäubung zurückzuführen sind. Wir werden daher keinen Fehlschluß machen, wenn wir die Färbung der Aleuronschicht bei unseren Kulturpflanzen mit dem analogen Vorkommen derselben schon bei einzelnen Sippen der Wildformen in eine stammesgeschichtliche Beziehung bringen.

Nach meiner Meinung dürfte es nicht notwendig sein die Blaufärbung des Weizens auf eine Bestäubung mit dem Roggen zurückzuführen. Bei den zahlreichen Rückbastardierungen meiner aller-

dings in  $F_1$  sterilen WR-Bastarde, sowie des fertilen WRB von RIMPAU mit gut grünkörnigen Roggenrassen wäre doch das Auftreten von Xenienfrüchten in den nächsten Generationen wahrscheinlich gewesen. Ferner wurde in diesem Jahre eine bereits 5 Jahre lang konstant gebliebene blaukörnige Weizenform mit einer gut grünkörnigen Roggenrasse bastardierte und zeigten die wenigen (8), allerdings zum Teil nicht gut ausgebildeten Körner ein Farbloswerden der Aleuronschicht des sonst blaukörnigen Weizens. Es würde demnach, wenn sich bei Wiederholung dieses Versuches in größerem Maßstabe dieses Resultat wiederholen sollte, der Schluß berechtigt sein, daß der Anthokyanfaktor im blauen Weizen mit dem im Roggen nicht identisch ist. Diese Beobachtungen veranlassen mich die Blaufärbung der Aleuronschicht des von KATTERMANN bearbeiteten, blaukörnigen Weizens nicht auf den Einfluß einer spontanen Kreuzung mit Roggen, sondern auf den Einfluß einer *Aegilops*-Form mit bläulichem Endosperm zurückzuführen.<sup>1)</sup>

Es lag nahe zur Entscheidung, ob Roggen oder doch *Aegilops* bei der Entstehung des „blaukörnigen“ Weizens mitgewirkt hat, die zytologische Untersuchung heranzuziehen. Zeigt doch die Weizen-*Speltoid*form 21/42, hingegen Roggen 7/14 und *Aegilops ovata* 14/28 Chromosomen. Bei reinlichem Erhaltenbleiben beider elterlichen Garnituren wäre sonst für einen additiven Bastard *Speltoid*  $\times$  Roggen die Kernschleifenzahl  $21 + 7/42 + 14$  bzw. 28/56, für einen additiven Bastard *Speltoid*  $\times$  *Aegilops ovata*  $21 + 14/42 + 28$  bzw. 35/70 zu erwarten. Die Untersuchung KATTERMANNs hat allerdings keine klare Entscheidung zwischen diesen beiden Möglichkeiten geliefert, indem eine Diploidzahl von 36 bis 44, unter Vorherrschen von 42 gefunden wurde: letztere würde einfache numerische Gleichheit mit dem *Speltoid* 21/42 bedeuten, also komplette Genophthise der Chromosomengarnituren der Vaterart mit eventueller patrokliner plasmatischer Nachwirkung (im Sinne von A. TSCHERMAK-SEYSENEGG). Da KATTERMANNs Befund ebensowenig gegen wie für eine Beteiligung von Roggen spricht, bleibt natürlich auch *Aegilops* möglich: „pater incertus“! Es könnte höchstens sein, daß einmal andere Produkte der beiden Kombinationen von analoger Kornfarbe ein klareres Resultat ergeben.

#### Literaturverzeichnis

- (1) E. Tschermak-Seysenegg. Ber. d. D. Bot. Ges. 47, 253 (1929) und 48, 400 (1930); (mit H. BLEIER) ebenda, 44, 110 (1926); Zeitschrift f. ind. Abst. u. Vererb. L. 56, 180, 1933, vergl. ebenda H. v. BERG 57, 342, 1934 und 58, 94, 1934.
- (2) G. Kattermann. Zeitschr. f. Ind. Abst. u. Vererb. Bd. 60. 1932; Zeitschr. f. Züchtung Reihe A Heft 4, Bd. 17 1932. S. 413-446. Prakt. Bl. f. Pflanzenbau und Pflanzenschutz 14, 266, 1936.

1) KATTERMANN ist nach brieflicher Mitteilung inzwischen selbst von der Heranziehung des Roggens abgekommen.

## Notes on the Anatomy of Variegated Leaves

By

**M. Sakisaka**

Tokyo Imperial University

(With One Plate)

In the present work a number of plants with variegated leaves, which are commonly found with us, were submitted to anatomical observations. Variegation is one of the leaf characters of which the Japanese have been very fond of since olden times and it shows great variation in its manifestations which have been classified in various ways by many horticulturists. It is not the object of this work, however, to classify the types of variegation viewed from their external appearances, but to elucidate the anatomical structure of such leaves. Such attempts on the anatomy of variegated leaves have been undertaken by many authors, such as Dalitzsch (1886), Hassack (1886), Sou Jou Tsinen (1923), Funaoka (1924), Küster (1927), and others. Küster (1927) giving a full list of former investigations, tried to classify the variegated leaves from the anatomical view point. His classification does not always conform with the results of the writer's observations, so that a provisional classification based on anatomical observations, so far as the writer has carried them out is proposed here.

The variegated leaves were hand-sectioned and mounted in water for microscopical observations. Photomicrographs were taken by using Zeiss ocular K. 15 and Leitz objective 3.

From the view point of the distribution of chloroplasts in the variegated leaves, the plants studied have been divided into six groups as follows:

**I. *Ternstroemia* type.** In *Ternstroemia japonica* of mediovariegate form, the boundary between the variegated and green areas seen from the outside is distinct. This is caused by the fact that in the first outer layer of the palisade tissue in the white region the chloroplasts are entirely absent in the cells adjacent to those in which the chloroplast-containing green tissue is found.

Example: *Ternstroemia japonica* (Fig. 1).

**II. *Ficus* type.** In *Ficus elastica* of margivariegate form two layers of palisade cells which lie under the colourless water storage tissue contain a considerable number of chloroplasts as seen in Fig. 2. The

difference in quantity of the chloroplasts in the second layer, however, causes the external distinction between the variegated and green areas of the leaf.

Example: *Ficus elastica* (Fig. 2).

**III. *Camellia* type.** In *Camellia japonica* of albomarginate form, two palisade layers of the green area contain chloroplasts, while those of the variegated area do not show so many chloroplasts as those of the former. The vascular bundle is always situated at the border region of the two areas (Figs. 3, 4).

In *Osmanthus aquifolium* it is noticed that the variegated portion of the leaf becomes thinner than in the green portion (Fig. 4); this is due to the decrease in size of the cells, and not to the numerical reduction of the cells.

Example: *Camellia japonica* (Fig. 3), *Osmanthus aquifolium* (Fig. 4), *Hedera japonica*, *Petasites japonicus*, *Polygonum orientale* var. *pilosum*, *Hydrangea opuloides* var. *Azissai*, *Elaeagnus pungens*.

**IV. *Daphne* type.** Though in *Daphne odora* of albomarginate form, the variegated area is seen sharply discriminated from the green area when viewed from the outside, this is not proved to be so microscopically in sections, because the numerical decrease of chloroplasts takes place gradually within a very limited portion from the green part of the palisade layer to the variegated part in which the chloroplasts are not generally found. It is sometimes observed that the variegated portion is thinner than the green portion, which is also due to the decrease in size of the cells as in the case of *Osmanthus aquifolius*.

Example: *Daphne odora* (Fig. 5), *Aucuba japonica* (Fig. 6), *Pieris japonicum* (Fig. 7), *Euonymus japonicus* (Fig. 8), *Ilex latifolia*, *Eurya ochnacea*, *E. japonica*.

**V. *Rhodea* type.** In the variegated area of *Rhodea japonica* of mediovariegate or albomarginate form, the chloroplasts are not included generally in the parenchymatous mesophyll in which the palisade tissue is not differentiated. In sections groups of cells containing chloroplasts are found scattered in such colourless tissue. The groups of green cells represent the green area of the leaf. This is characteristic of the monocotyledonous leaves.

Examples: *Rhodea japonica* (Fig. 9), *Aspidistra daibuensis* (Fig. 10), *Liriope graminifolia*, *Ophiopogon Jaburan*, *Hosta japonica*.

**VI. *Arundo* type.** In *Arundo donax* of albomarginate form, all the mesophyll cells in the green area contain chloroplasts, but in the portion corresponding to the variegated area no chloroplasts are found. This type is also characteristic of the monocotyledonous leaves.



Example: *Arundo donax* (Fig. 11), *Hemerocallis aurantica*, *Isachne globosa*, *Phalaenopsis Aphrodite*, *Zingiber Mioga*, *Phormium tenax*, *Acorus gramineus*.

In all, twenty-six species and two varieties were here listed, of which sixteen are dicotyledons belonging to four types (I, II, III, and IV), while fourteen are monocotyledons belonging to types V and VI. Besides a number of plants with variegated leaves have also been investigated. They seem to be of types either intermediate between, or of uncertain classification in the types mentioned above.

The writer wishes to express his sincere thanks to Prof. Miyake and Prof. Ogura of Tokyo Imperial University for their helpful criticism and advice. Thanks are also due to Mr. K. Suzuki for his kindness in preparing the photomicrographs.

Botanical Institute, Faculty of Agriculture,  
Tokyo Imperial University

#### References

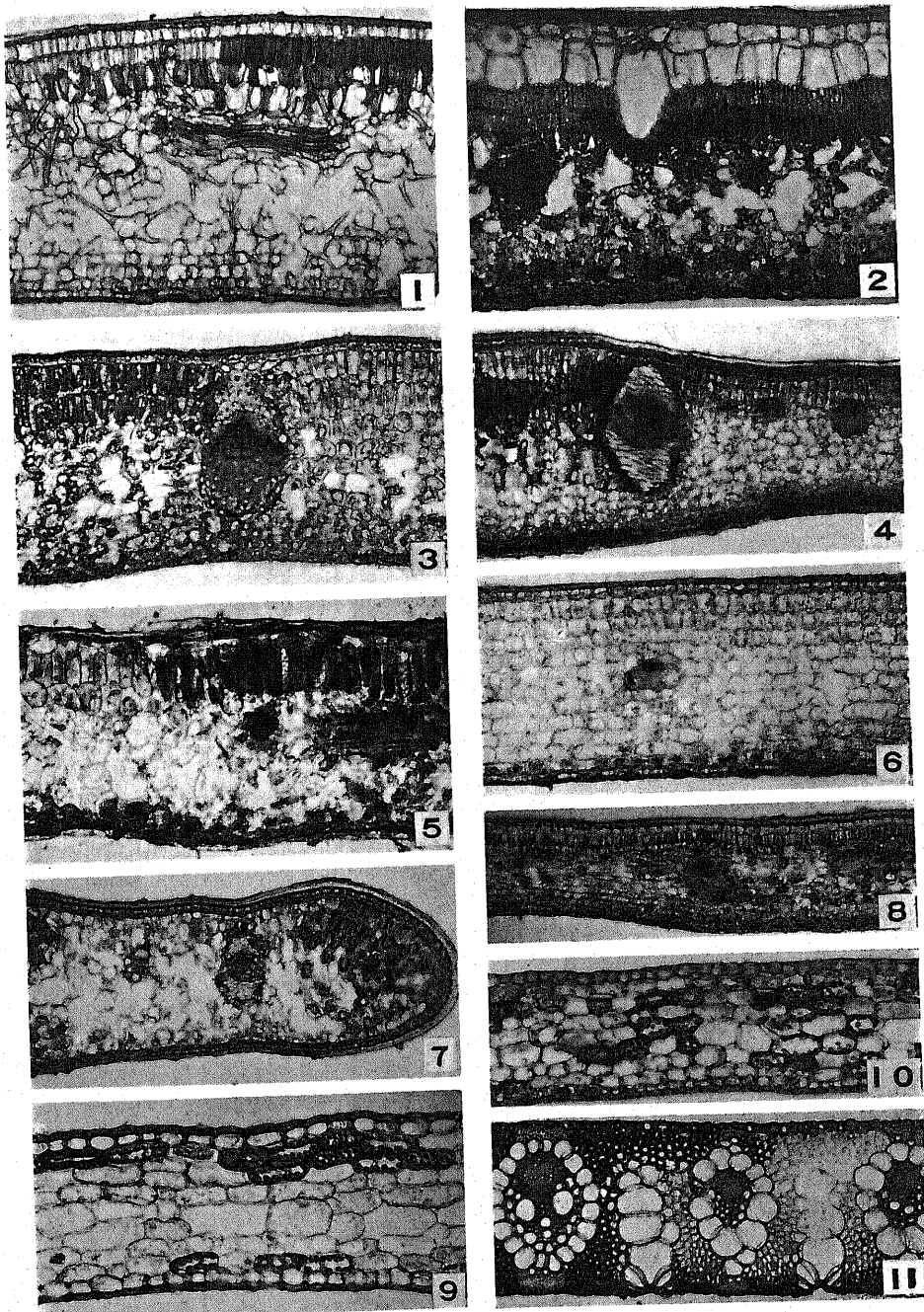
- Imai, Y. 1935. The structure of albomarginata and medioalbinata forms. Jour. Genet., Vol. 31.  
Küster, E. 1927. Anatomie des panaschierten Blattes. Linsbauer's Handbuch d. Pflanzenanat. Vol. 8.

#### Explanation of Plate 35

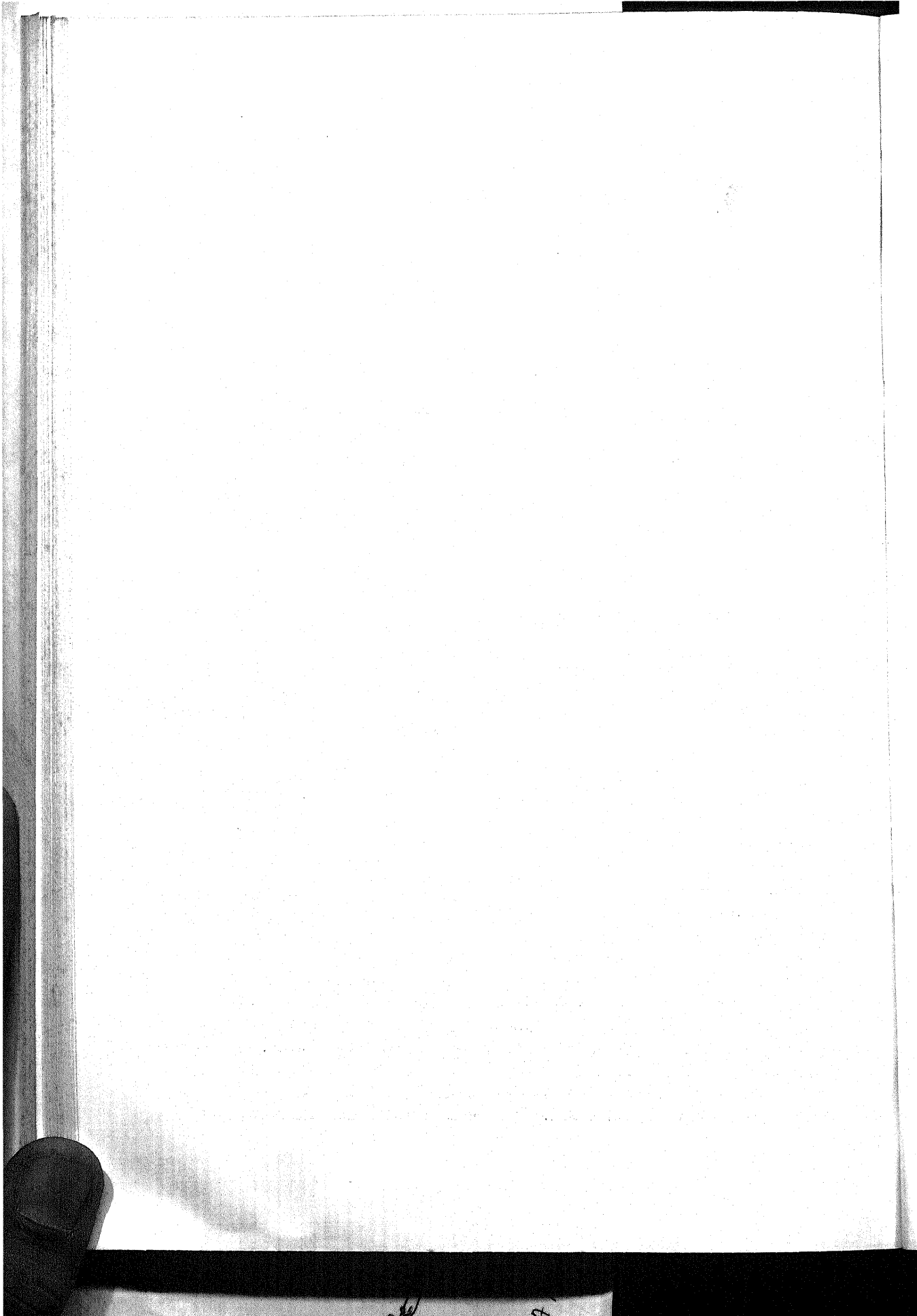
Photomicrographs of cross sections of variegated leaves. Magnification:  $\times$  ca 65.

- Fig. 1. *Ternstroemia japonica*
- Fig. 2. *Ficus elastica*
- Fig. 3. *Camellia japonica*
- Fig. 4. *Osmanthus aquifolium*
- Fig. 5. *Daphne odora*
- Fig. 6. *Aucuba japonica*
- Fig. 7. *Pieris japonicum*
- Fig. 8. *Euonymus japonicus*
- Fig. 9. *Rhodea japonica*
- Fig. 10. *Aspidistra daibuensis*
- Fig. 11. *Arundo donax*





Sakisaka: Notes on the Anatomy of Variegated Leaves



## The Internal Structure of Chromosomes—A Statement of Opinion

By

C. L. Huskins

McGill University, Montreal

With few exceptions the path of progress in cytology has led to increasingly complex views of chromosome structure and behaviour. The greatest single observational step towards complexity was probably that made by Professor Fujii (1926) when he discovered the "double spiral" nature of chromosomes. It therefore seems fitting that, in a volume published in his honour, spiral structure should be discussed and points at issue today be clarified. A clarification is particularly desirable because a number of different and apparently somewhat opposed current views on spiral structure may, it appears, not really be fundamentally different. I shall attempt to show how some misunderstandings have probably arisen. Only the minimum number of references will be cited; I shall attempt a personal discussion, not a review, especially since Kaufmann (1936) has just published one.

As is well known, Baranetzky (1880) first discovered a spiral chromonema within the chromosome. His views did not, however, gain general acceptance. From 1880 to 1925 only three cytologists appear to have described spiral structure with any degree of detail or assurance; they were Bonnevie (1908, 1911), Schneider (1910) and Vejdovsky (1912). Kaufmann (1925) found "double spiral bands" in both somatic and meiotic chromosomes. Fujii stated that meiotic chromosomes have a "spiral within a spiral" structure. Fujii's work did not, however, become directly known to cytologists in general owing to its having been presented in Japanese in a Journal which is available in few, if any, occidental libraries. The dissemination of his views has depended on the numerous publications of Ishii, Sakamura, Kuwada, Nakamura, Shinke and other Japanese workers, dating from 1931 to the present. According to the view of these workers there is a small gyred spiral running along the larger gyred spiral chromonemata. These have been described as primary and secondary spirals respectively and also *vice versa*. To avoid confusion they are now generally designated major and minor spirals. This structure has sometimes been described as a "double spiral", but this has led to confusion as Kuwada (1927) used this term to indicate that each chromosome major spiral

is composed of two closely appressed chromatid major spirals. It is concerning the existence or the form of the minor spiral that most disagreement occurs today. Nebel (1932) for instance has shown very *loosely* coiled *half-chromatid* minor spirals; Kuwada and Nakamura showed *in a diagram* (1933, text fig. 3) a very *tightly* coiled *chromatid* minor spiral. Their model is such as one would obtain if a spiral spring with closely appressed gyres, (such as is commonly used on screen doors) were itself coiled rather loosely into a spiral with its gyres about six times the diameter of the original spring. Huskins and Smith (1935) could find no evidence in *Trillium* for this *diagrammatic* interpretation, which involves the presence of only four longitudinal strands (chromatids) in a bivalent. We found eight strands and a waviness which we considered compatible with the minor spiral interpretation of Nebel, but which *seemed* definitely different to the minor spiral concept of Kuwada and his co-workers, as we interpreted their description. Discussion of the problem with a number of cytologists has, however, indicated that the disagreement is perhaps superficial only. What appears in many of Kuwada's and other Japanese workers' photographs to be a closely coiled unitary *chromatid* minor spiral running along the length of the major spiral, can be seen at some points to be two *half-chromatid* spirals. Very small-gyred *chromatid* spirals shown in some photographs and drawings of other workers, *cf.* Darlington (1935 fig. 7 and p. 40) are fairly clearly due to the drawing out of the major spiral, as is proved for a certain small spiral in *Trillium* (1935, text fig. 8). These should not be confused with the minor spiral. Further, it is still questionable whether the small-gyred coils of somatic and homoeo-typic mitosis should be homologised with the minor spiral; data bearing on this problem will be published later.

By their ingenious ammonia treatments Kuwada and his co-workers have shown that the major spiral can be uncoiled and minor coils are stated to be much clearer in drawn-out parts than along major coiled regions. Their photographs of chromosomes after such treatment (e.g. 1933, fig. 14 and 15) show regions that *can* be interpreted as *closely* coiled *chromatid* minor spirals, i.e., as involving four strands per bivalent, as in their diagram Text fig. 3. Alternatively, however, they can be interpreted as showing two parallel waved or coiled chromonemata in each chromatid—an interpretation which involves the existence of eight strands. Correspondence with various cytologists interested in spiral structure showed that some of them had interpreted the photographs in one way and some in the other. I had assumed that the text figure was to be taken as a more or less exact model; hence the statement (1935) that our observations on

*Trillium* did not agree with the major and minor spiral interpretation of Japanese cytologists. Kuwada (1935) has recently confirmed the occurrence of eight chromonemata in *Trillium* bivalents, and Kuwada and Nakamura (1935) have recently found that the anaphase chromosomes of the second meiotic division in *Tradescantia* are bipartite. It is evident, therefore, that they cannot now be accepting their 1933 text fig. 3 as a precise model.<sup>1)</sup> Whether they ever did so seems doubtful. They are not explicit on the point in that paper; presumably they were chiefly concerned in supporting Fujii's minor spiral interpretation, which they, quite rightly, considered the important issue at that time. Darlington (1935) has, however, adopted their *diagrammatic* interpretation and neglected the abundant evidence, including that of these same authors, that metaphase bivalents are at least 8-partite. His Fig. 21 shows three interpretations made from one *Tradescantia* preparation, and all agree in showing compact coils and only two strands per "dyad"; the latter is in fact a cardinal feature of several of his hypotheses.

It is certainly necessary, if clear thinking is to follow, that a clearer definition of present viewpoints on spiral structure should be reached. Differences in the material studied may, of course, be one source of confusion. For instance, the chromatid major coils lie widely separate in *Trillium*, but in *Tradescantia* they often lie so close together that there seem to be only two coils per bivalent. The minor spirals may show a similar difference in some cases. Evidently, however, such differences are responsible for only part of the difficulty in reaching a common interpretation. Kaufmann (1936), for instance, presents a diagram of major and minor coils in *Tradescantia* which is somewhat like our interpretation of *Trillium*, but very different from the *diagrams* of *Tradescantia* structure presented by Kuwada and Nakamura (1933) or Darlington (1935).

A second point on which there is some disagreement is that of changes of direction occurring in the major spiral. Nebel (1932) reported changes on either side of the attachment region in *Tradescantia*, but very rarely elsewhere. Darlington (1932, 1935) argues from *a priori* assumptions (see Sax 1936) that they cannot occur elsewhere, with the possible exception of secondary reversals at the ends. They have, however, been shown to occur at various points in *Gasteria* by Taylor (1925), in *Secale* by Sax (1930), in *Lilium* by Iwata (1935), in *Trillium* by Huskins and Smith (1932, 1935) and

1) Professor Kuwada has recently confirmed this in a personal communication. He and his colleagues now see a "geminus as 8-stranded but 4-partite (4 chromatids)" as we do. What I have here called the "half-chromatid" minor spirals may, he emphasises, be so associated that in some regions they look like chromatid minor spirals.



Matsuura (1935). Our work (1935) showed that in *Trillium* there was probably a facultative relationship between changes of direction and chiasmata. Unpublished work by A. W. S. Hunter, H. Newcombe and myself confirms this both by direct observation and experiment. To mention only one point, in asynaptic *Trillium* changes of direction are rare and confined almost entirely to the vicinity of the attachment, see Figs. 11 and 12. The difference between *Tradescantia* and *Trillium* with regard to the position and frequency of the changes of direction is, as we (1935) suggested, almost surely due to differences in time of the establishment of coiling relative to terminalization.

The evidence for multiplicity of chromonemata in both somatic and meiotic chromosomes appears now to be overwhelming (see Kaufmann 1936). The older view that chromosomes "split" during the "resting stage" immediately prior to the division in which their halves are separated from each other must almost as surely go into discard as the still earlier idea that they "split" at metaphase and separate immediately afterwards at anaphase. Observational evidence for double somatic anaphase chromosomes and octopartite meiotic bivalents has been cited. Nebel (1932), Stebbins (1935) and Goodspeed, Uber and Avery (1935) go further in their claims of quadripartite somatic anaphase and Nebel (1936) presents evidence for quadripartite leptotene threads and octopartite meiotic first anaphase chromosomes. I have seen the somatic chromosome preparations of Nebel and Stebbins (Genetics Society of America 1934) and am convinced that they are right for at least some cells of some organisms, but we have not yet been able to obtain convincing evidence of more than two chromonemata in somatic and four in meiotic first anaphase chromosomes in our own materials in this laboratory. Bauer (1936) reports 100-400 chromonemata in the "paired" salivary gland chromosomes of certain Chironomidae; it is generally agreed that 16 are most commonly found in *Drosophila*. There is much more, though less definite, evidence that the number of chromonemata is to some extent variable. The demonstration of White (1935) and others that sex chromosomes and autosomes of the same complement differ in their time of splitting is collateral evidence supporting this view. I have found the chromosome split (not only the chromonema double) in root-tip and microspore mitotic anaphases in a few rare cases.

Experimental evidence on the number of chromonemata per chromosome and the time of "splitting" has been sought in X-ray experiments by a number of workers. Mather and Stone (1933) found only breaks of whole chromosomes. Huskins and Hunter (1935) found chromatid breaks which they considered proved second meiotic

anaphase chromosomes to be longitudinally double. Marshak (1935) found configurations indicating that the chromosomes are at least double before synapsis. White (1935) found some chromatid breaks in X-rayed *Locusta*. Nebel (1936) found that with low dosage chromatid breaks occur indicating that the chromosomes are double before synapsis. With higher dosages he obtained some half-chromatid breaks, which support his morphological observation that chromosomes are quadripartite structures prior to synapsis. Gustafsson (1936) finds a few chromatid breaks, but argues that they do not necessarily prove doubleness at the time of irradiation; the capacity of the "fibril" to reproduce itself may have been impaired, he considers, and he continues to support the view that somatic anaphase chromosomes are longitudinally single structures. This interpretation ignores genetic data on *Drosophila* such as those of Patterson (1933) and Moore (1934). The former assumed from his data that the chromosomes were double in about one in seven of the sperm irradiated. The latter considers X-ray mosaic data prove doubleness but that whole chromosome breaks do not prove singleness; he suggests that effects on the matrix are responsible for chromosome breaks. The first part of this conclusion is the same as that reached by Huskins and Hunter (1935) from X-ray experiments on *Trillium*. I do not, however, consider it necessary to invoke the aid of the matrix to explain chromosome breaks. The *Drosophila* experiments of Demerec (1935 and unpublished) and others show that an X-ray "hit" usually "knocks out" a number of longitudinally adjacent salivary gland chromosome bands. The chromonemata are for the most part extremely closely appressed even in those organisms where multiplicity of strands can most clearly be demonstrated. The *a priori* expectation from the *Drosophila* data is that chromosome breaks will be more frequent than chromatid breaks, even if all chromosomes are longitudinally subdivided structures at all stages. If the degree of subdivision may differ in different tissues or different organisms, that of course gives a further alternative explanation for differences in the frequency of chromosome and chromatid breaks found in different X-ray experiments. At any rate, I see no reason to change the opinion (Huskins and Hunter 1935) that chromatid breaks are almost decisive evidence of doubleness at the time of irradiation whereas chromosome breaks are no evidence at all of singleness. Cumulatively the observational and experimental evidence, both cytological and genetical, is overwhelmingly in favour of the point of view that chromosomes are at least longitudinally double structures. The adherents of the older view have, like all conservative forces, furthered real progress by checking too uncritical advances, but the



time now seems definitely to have come when they must move ahead or be classified merely as "die-hards". If this is so, two of the most stimulating of working hypotheses, Darlington's "precocity theory" and Belling's (1933) theory of crossing-over must now be abandoned. My own (1933) "mitosis-meiosis" hypothesis, which was an effort to modify the precocity theory so that it could embrace the evidence of the "tertiary" split, the apparent singleness of leptotene threads and the doubleness of somatic anaphase chromosomes, has already preceded them into limbo. It may be added, however, that the "effective" singleness of the kinetochore, *cf.* Schrader (1936), and the "effective" unity of the chromatid regardless of the number of chromonemata it contains, renders some conclusions based initially on the precocity theory still valid, even though the primary postulates of that theory are invalid—which attests to the service it has rendered as a *working hypothesis*.

In closing these remarks, however, I should like to express the opinion that all attempts to correlate meiotic and mitotic phenomena on purely morphological bases are foredoomed to failure. A "physiological" unity is not at all necessarily a morphological one, and subdivision of the chromonema may bear little direct relationship to either pairing or "splitting" of the chromosomes, as salivary gland studies particularly attest. Further, observable doubleness or singleness may, apart altogether from artefact, be a reversible process. The "tertiary" split which is so often very clear at first metaphase in *Trillium* is not ordinarily visible at second anaphase (Huskins and Smith 1935); the leptotene threads of *Trillium* are, as we recorded, unmistakably single optically after all of the many fixing and staining methods we have tried, but I have more recently seen very clear indications of doubleness in pre-leptotene cells. Today it seems that physiological hypotheses of the relationship between mitosis and meiosis, such as those of Stebbins (1935) and Gustafsson (1936) are most likely to be of service; it will be unfortunate if they are too closely tied, as the latter's is at present, to morphological interpretations. The complex modern interpretations of chromosome structure, which so largely date from the time of Professor Fujii's work in 1926, cannot be fitted into simple "valency" schemes.

#### References

- Baranetsky, J., 1880. Die Kernteilung in den Pollenmutterzellen einiger Tradescantien. Bot. Zeit. 38: 241-247; 265-274; 281-295.  
Bauer, H., 1936. The structure of the salivary gland chromosomes in Chironomidae. Am. Nat. 70: 37.  
Belling, J., 1933. Crossing-over and gene arrangement in flowering plants. Genetics 18: 388-413.

- Bonnevie, K., 1908. Chromosomenstudien I. Arch. Zellforsch. 1: 450-514.  
— 1911. Chromosomenstudien III. Arch. Zellforsch. 6: 190-253.
- Darlington, C. D., 1932. *Recent advances in cytology* pp. 559. Blakiston.
- Darlington, C. D., 1935. The internal mechanics of the chromosomes I, II, and III. Proc. Roy. Soc. London B. 118: 33-96.
- Demerec, M., 1935. Cytogenetic evolutionary processes and their bearing on evolutionary theory. Amer. Nat. 69: 125-138.
- Fujii, K., 1926. Recent progress in cytology and its method of investigation. (Japanese). Report of Jap. Ass. Adv. Sci. 2: 373-415.
- Goodspeed, T. H., Uber, F. M. and Avery, P., 1935. Application of the Altmann freezing-drying technique to plant cytology. III. Chromosome structure in *Lilium longiflorum*. Univ. Cal. Publ. Bot. 18: 33-44.
- Gustafsson, A., 1936. The different stability of chromosomes and the nature of mitosis. Hereditas 22: 281-335.
- Huskins, C. L., 1933. Mitosis and meiosis. Nature 132: 62-63.  
— et al. 1932. Demonstrations of chromosome structure. Proc. VI. Int. Genetics Congress, vol. 2: 392, 3, and 6.  
— and Hunter, A. W. S., 1935. The effects of x-radiation on chromosomes in the microspores of *Trillium erectum* Linn. Proc. Roy. Soc. London B. 117: 22-23.  
— and Smith, S. G., 1935. Meiotic chromosome structure in *Trillium erectum*. Ann. Bot. 49: 119-150.
- Iwata, J., 1935. Chromosome structure in *Lilium*. Mem. Coll. Sci., Kyoto Imp. Univ. B. 10: 275-288.
- Kaufmann, B. P., 1925. The existence of double spiral bands and a "bouquet" stage in *Tradescantia pilosa* Lehm. Am. Nat. 59: 190.  
— 1936. Chromosome structure in relation to the chromosome cycle. Bot. Rev., vol. 2, no. 11: 529-554.
- Kuwada, Y., 1927. On the spiral structure of chromosomes. Bot. Mag. (Tokyo) 41: 100-109.  
— 1935. Behaviour of chromonemata in mitosis. Cytologia 6: 308-313.  
— and Nakamura, 1933. Behaviour of chromonemata in mitosis. I. Mem. Coll. Sci., Kyoto Imp. Univ. B. 9: 129-139.  
— and — 1935. Behaviour of chromonemata in mitosis. VI. Cytologia 6: 314-319.
- Mather, K. and Stone, 1933. The effect of x-radiation upon somatic chromosomes. Jour. Genetics 28: 1-24.
- Marshak, A. G., 1935. The effect of x-rays on chromosomes in different stages of meiosis. Journ. Gen. Physiol. 19: 179-198.
- Moore, W. G., 1934. A comparison of the frequencies of visible mutations produced by x-ray treatment in different developmental stages of *Drosophila*. Genetics 19: 202-222.
- Nebel, B. R., 1932. Chromosome structure in *Tradescantiae*. I and II. Zeits. f. Zellf. Mikro. Anat. 16: 251-304.  
— 1936. Chromosome structure X. An x-ray experiment. Genetics 21: 605-614.
- Oura, G., 1936. A new method of unravelling the chromonema spirals. Zeits. wiss. Mikro. 53: 36-37.
- Patterson, J. T., 1933. The mechanism of mosaic formation in *Drosophila*. Genetics 18: 32-52.
- Sax, K., 1930. Chromosome structure and the mechanism of crossing over. Jour. Arn. Arb. 11: 193-220.  
— 1936. Chromosome coiling in relation to meiosis and crossing over. Genetics 21: 324-338.
- Schrader, F., 1936. The kinetochore or spindle fibre locus in *Amphiuma tridactylum*. Biol. Bull. 70: 484-498.
- Stebbins, G. L., 1935. Chromosome structure and the mechanism of meiosis in plants. Amer. Nat. 69: 81.
- Taylor, W. R., 1925. Cytological studies on *Gasteria*. II. Amer. Jour. Bot. 12: 219-223.

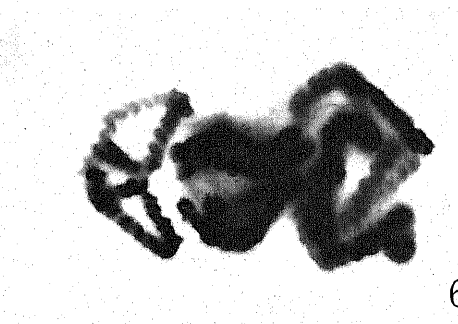
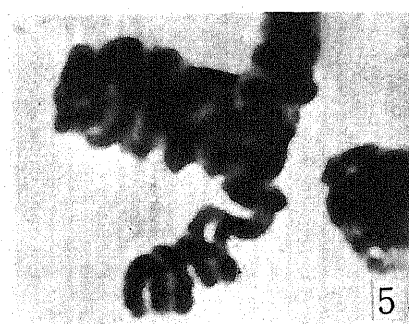
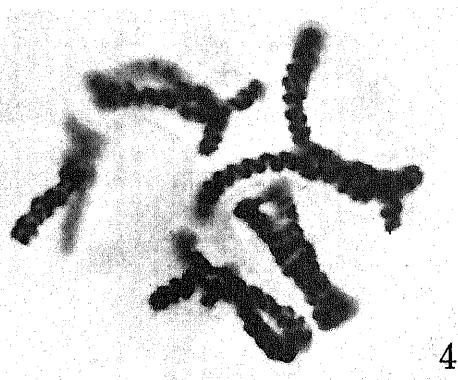
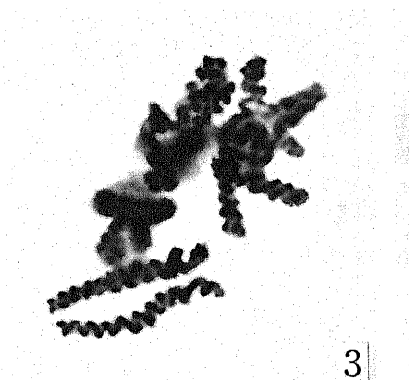
- Vejdovsky, F., 1912. *Zum Problem der Vererbungsträger*. Kön. Böhm. Ges. Wiss. Prag. pp. 1-184.
- White, M. J. D., 1935. The effects of x-rays on mitosis in the spermatogonial divisions of *Locusta migratoria* L. Proc. Roy. Soc. London B, 119: 61-84.

#### Explanation of Plates 36 and 37

Meiotic chromosomes of *Trillium erectum*. Smear preparations dessicated 20-30 seconds, fixed in La Cour's 2 Bd or S<sub>2</sub>, and stained with iodine gentianviolet.

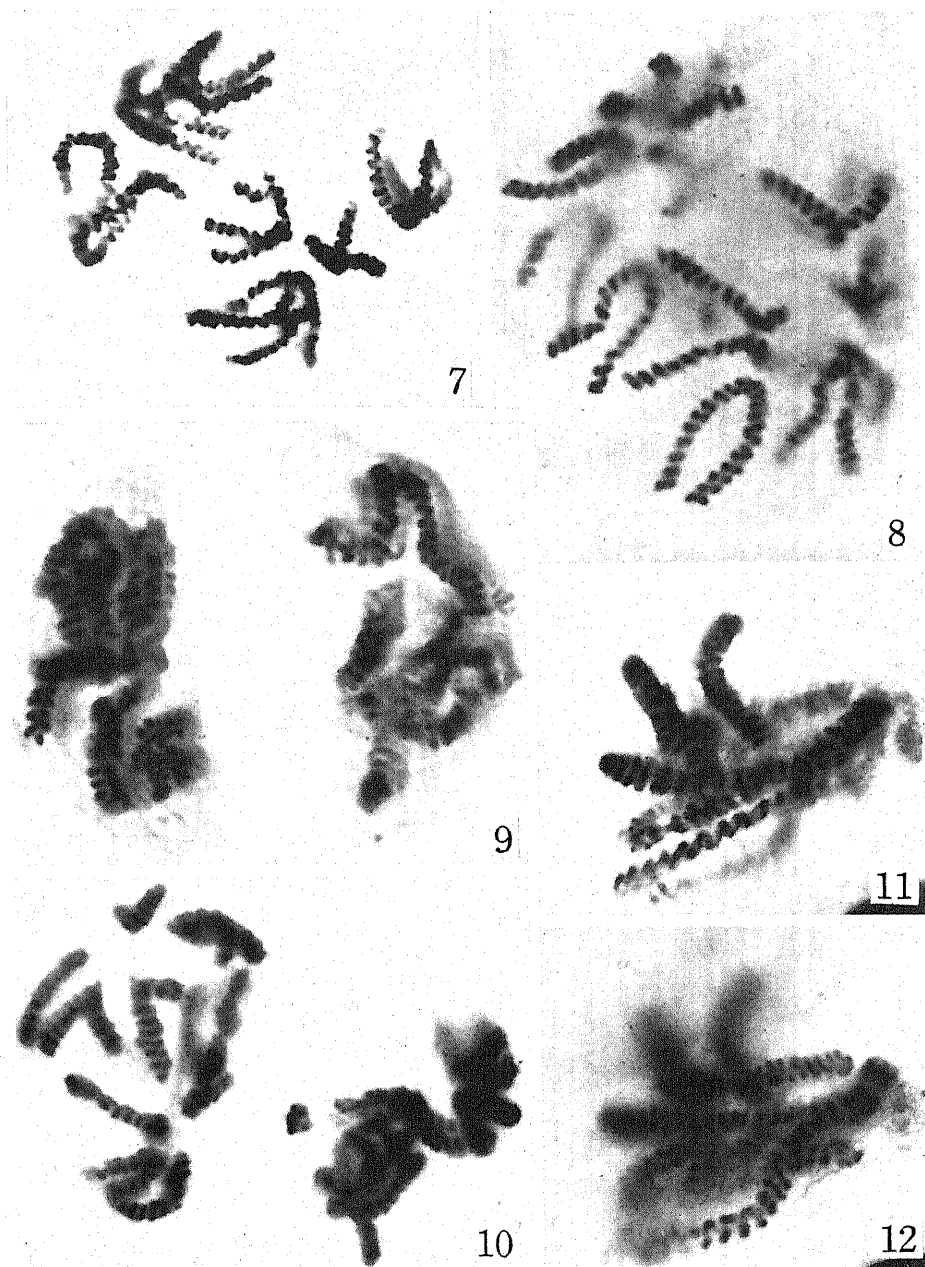
- Fig. 1. Early metaphase showing the tertiary split, especially in chromosome at 3 o'clock. ca. 1800×.
- Figs. 2-4. First metaphases showing coiling, the nature of the attachment region, and pairing with and without chiasmata. ca. 1800×.
- Fig. 5. Composite photograph of two optical levels showing major spiral; note the wavyness of its outline. ca. 3600×.
- Figs. 6-8. First anaphase; side view, tangential view, and polar view respectively. Note changes of direction of coiling. ca. 1800×.
- Fig. 9. Second metaphase; Fig. 10: second anaphase. Note similarity of major coils to those of the first anaphase. ca. 1800×.
- Figs. 11 and 12. Metaphase in asynaptic *Trillium*; two optical levels of the same cell. ca. 1800×.

I am indebted to Mr. S. G. Smith for assistance with the photographs. Figs. 1-10 are new photographs or improved prints of figures already published in our joint paper (1935). Figs. 11 and 12 are from work in progress by A. W. S. Hunter and myself.



Huskins: The Internal Structure of Chromosomes—A Statement of Opinion





Huskins: The Internal Structure of Chromosomes—A Statement of Opinion





## Chromosomenketten beim Rassenbastarde des Seidenspinners *Philosamia cynthia*

### (Zytologische Untersuchungen am Seidenspinner und seinen Verwandten. III)

Von

Eisaku Kawaguchi

#### I. Einleitung

Über *Philosamia cynthia* DRURY wurden bis jetzt drei zytologische Arbeiten von DEDERER veröffentlicht. Diese Verfasserin hat über die Art, die in Amerika gefunden wird, Untersuchungen angestellt. Bei dieser Art stellte sie erst in der Spermatogenese und dann nachher in der Oogenese die Zahl von 13 haploiden Chromosomen fest (1907, 1915). Ferner hat sie über einen interessanten Fall in der Spermatogenese bei einigen Kulturen im Laboratorium berichtet, wobei Schwankungen der Chromosomenzahl gefunden wurden (1928). Unter zwei von vier Kulturen fand sie einige Reihen mit 12 Chromosomen und bemerkte, daß diese Chromosomenaberration auf die Assoziation zweier Chromosomen zurückzuführen ist. In einer anderen Reihe derselben Kulturen sind einige Individuen gefunden worden, die in einem und demselben Hoden Spermatozyten mit 13 bzw. 14 Chromosomen besaßen. Als Ursache der Erhöhung der Chromosomenzahl hat sie die Fragmentation eines Chromosoms angenommen, wenn sie auch nicht erkennen konnte, welches Chromosom fragmentiert wurde.

Das Ziel meiner Untersuchungen bestand in der Erforschung der Chromosomengarnituren bei *Philosamia cynthia*, die je nach den geographischen Rassen verschieden gefunden wurden, und ferner des Verhaltens der Chromosomen bei ihren Bastarden. Während einer zytologischen Untersuchung der Spermatogenese ihres Bastards wurden eigentümliche Chromosomenverkettungserscheinungen in Diakinese und Metaphase der beiden Reifeteilungen entdeckt. Auf Grund meiner Befunde machte ich den Versuch, die Prozesse, wodurch die Veränderung der Chromosomengarnitur einer Rasse im Laufe der phylogenetischen Entwicklung stattfindet, klar zu stellen und dazu einen Schluß auf die Ursache der Chromosomenschwankung, die von Dederer beobachtet wurde, zu ziehen.

#### II. Material und Methode

Zu meiner Untersuchung gelangten die folgenden zwei verschiedenen geographischen Rassen: *Philosamia cynthia Walkeri*

FELDER und *Ph. c. Pryeri* BUTLER. Beide Rassen finden sich auf paläarktischen Gebieten, und zwar *Walkeri* verbreitet vom zentralen und nördlichen China bis zur südlichen Mandschurei; *Pryeri* dagegen bewohnt nur Japan.

*Walkeri* unterscheidet sich von *Pryeri* durch das außen schärfer begrenzte Distalband beider Flügel und durch die langen Medianzähne des proximalen Vorderflügelbandes (SEITZ, 1916).

Nach meinem Versuche wurden die gegenseitigen Bastardierungen sehr leicht durchgeführt, wenn auch viele  $F_1$ -Raupen leider an einer schweren Fleckenkrankheit zugrunde gingen und nur einige Männchen von einer Kreuzung der *Walkeri* ♀ mit *Pryeri* ♂ sich bis zu Puppen entwickelten, die alle zur zytologischen Untersuchung getötet wurden.

Die Hoden wurden aus den Puppen der zwei reinen Rassen, von denen eine direkt aus der Mandschurei gesandt und die andere in Fukuoka (Süd-Japan) und Kyoto (Mittel-Japan) gesammelt worden war, und ihres Bastards entnommen und sofort in CARNOYs Chloroform-Eisessig-Alkohol fixiert. Die Schnitte wurden  $8\mu$  dick gemacht und mit Eisenhämatoxylin nach HEIDENHAIN gefärbt. Als Fixations- und Färbungsmittel diente sonst noch Karminessigsäure.

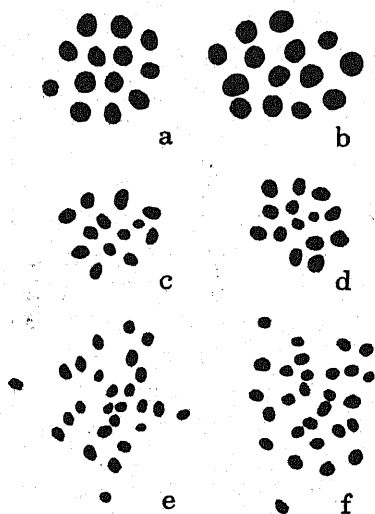


Abb. 1. Äquatorialplatten der Reifeteilungen. Vergrößerung 2400 mal. a-d. Eupyrene Spermatozyten. e, f. Apyrene Spermatozyten. a, b, e, f. Erste Reifeteilung. c, d. Zweite Reifeteilung. a, c. 13 Chromosomen aus *Walkeri*. b, d. 14 Chromosomen aus *Pryeri*. e. 26 Chromosomen aus *Walkeri*. f. 28 Chromosomen aus *Pryeri*.

### III. Beschreibung des Karyotypus von *Philosamia cynthia Walkeri*, *Ph. c. Pryeri* und ihrem $F_1$ -Bastarde

Eine eingehende Untersuchung der Spermatogenese bei *Philosamia cynthia Walkeri* bestätigte die Richtigkeit der von DEDERER veröffentlichten Chromosomengarnitur. Auf den guten Äquatorialplatten aus den ersten und zweiten Spermatozyten traten also 13 Chromosomen sehr deutlich hervor (Abb. 1, a, c und Taf. 38, 1, 2, 5). Ihre Gestalt ist bei allen fast kugelig, und der Unterschied in der Größe so gering, daß es fast unmöglich ist, die entsprechenden Chromosomen in verschiedenen Äquatorialplatten zu identifizieren.

Für *Pryeri* wurden mehr als 20 Männchen untersucht, von denen

die eine Hälfte in der Umgebung von Fukuoka und die andere in Kyoto gesammelt worden war. Auf allen Äquatorialplatten der ersten und zweiten Spermatozyten unterschieden sich die Karyotypen der *Pryeri* aus den zwei Gegenden in Japan weder nach der Chromosomenzahl noch nach ihrer Gestalt, dagegen die Karyotypen von *Walkeri* aus der Mandschurei ausnahmslos äußerst stark nach der Chromosomenzahl, die nämlich 14 anstatt 13 betrug (Abb. 1, b, d. Taf. 38, 3, 4).

An der Ecke der Follikeln der Hoden finden wir manchmal bei beiden Rassen anomale Spermatozyten, die sich wohl zu den apyrenen Spermatozoen entwickeln werden. In diesen Zellen findet man diploide Chromosomen wegen des Fehlschlagens der Konjugation der Chromosomen, und zwar 26 in *Walkeri* und 28 in *Pryeri* (Abb. 1, e, f).

Auf jeden Fall ist das Chromosomenverhältnis beider Rassen dem der von mir mitgeteilten zwei naheverwandten *Bombyx*-Arten (KAWAGUCHI, 1928) sehr ähnlich.

In der Spermatogenese des  $F_1$ -Bastards zwischen *Walkeri* und *Pryeri* fanden wir zu unserer Überraschung die dem *Bombyx*-Bastarde entsprechenden Verhältnisse nicht wieder. Äquatorialplatten der ersten Spermatozytentteilung zeigten nur 12 Chromosomen, darunter 10 in der Polansicht rund wie in den Elternrassen, zwei dagegen erschienen merkwürdig abnorm. Das eine abnorme Chromosom zeigte meistens eine birnenförmige Gestalt. Es machte den Eindruck, als ob ein kleines Chromosom sich an das normale bivalente angehängt habe. Das andere bestand aus einem langen, sich schlängelnden Element, das ungefähr drei- oder oft vierfach länger als die normalen Chromosomen war. Das wurde für eine Kette von vier univalenten Chromosomen gehalten, da es meistens aus vier Anschwellungen bestand. Abb. 2, a, e zeigen die drei Chromosomen-Garnituren mit den zwei abnormen Chromosomen im  $F_1$ -Bastarde.

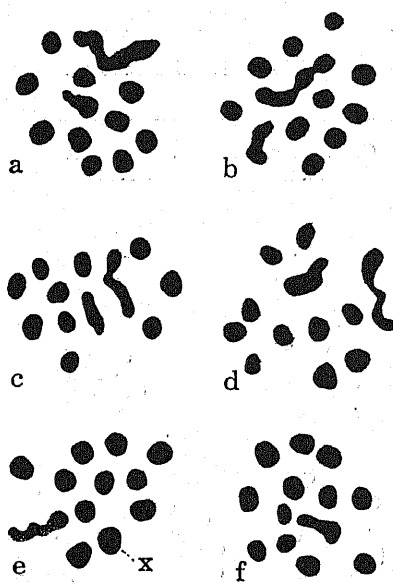


Abb. 2. Sechs Äquatorialplatten der ersten Spermatozytentteilung im  $F_1$ -Bastarde. Vergrößerung 2400 mal. a-e. 12 Chromosomen mit zwei Chromosomenketten, aber bei e steht die eine, mit X bezeichnet, senkrecht der Platte. f. Ausnahmsweise 13 Chromosomen mit einer Kette.

Die Chromosomenkette lag gewöhnlich horizontal, d.h. rechtwinklig zur Spindelachse auf der Äquatorialplattenebene, selten senkrecht, d.h. parallel zur Spindelachse, wie die Seitenansicht zeigte (Abb. 3). Sehr selten beobachtete man, daß die zwei normalen Bivalenten durch Fehlschlagen der Verkettung hervorgerufen wurden (Abb. 2, f).

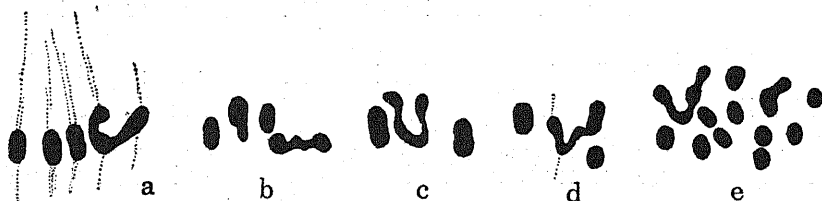


Abb. 3. Fünf Seitenansichten der Äquatorialplatten der ersten Spermatozytenteilung im  $F_1$ -Bastarde. Vergrößerung 2400 mal. a-d. Auf jeder ist nur eine lange Kette sichtbar. e. Zwei Ketten (Platte in etwas schiefer Winkel gesehen und gezeichnet).

Unter den Äquatorialplatten der zweiten Reifeteilung finden sich einige verschiedene Platten mit 12, 13 und 14 Chromosomen, darin eine bzw. zwei Chromosomenketten, die als zweigliedrig zu erkennen sind. Diese Beobachtungen stellt die Tabelle 1 zusammen, und die Zeichnungen davon werden in Abb. 4 gegeben.

Tabelle 1. Chromosomenzahl der zweiten Teilung der Spermatozyten des  $F_1$ -Bastards

Chromosomenzahl	Summe der Zellen	Zellenzahl		
		ohne Kette	mit 1 K.	mit 2 K.
12	5	0	3	2
13	10	4	3	3
14	2	1	1	0

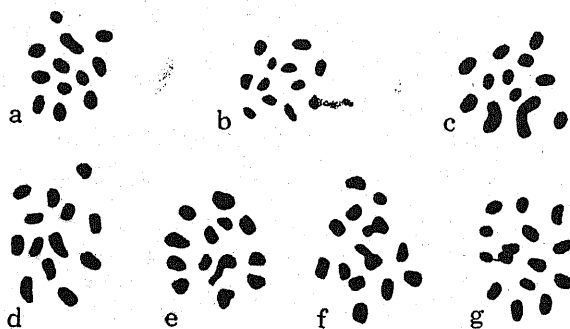


Abb. 4. Sieben Äquatorialplatten der zweiten Spermatozytenteilung in  $F_1$ -Bastarde. Vergrößerung 2400 mm. a-b. 12 Chromosomen mit einer Kette. c. 12 Chromosomen mit zwei Ketten. d. 13 Chromosomen ohne Kette. e. 13 Chromosomen mit einer Kette. f. 13 Chromosomen mit zwei Ketten. g. 14 Chromosomen mit einer Kette.

Um die Entstehung dieser zwei abnormen Chromosomenketten in der ersten Teilung mit derjenigen der anderen normal konjugierten Chromosomen zu vergleichen, wurden die Tetraden-Gestalten in Diakinese untersucht. In der früheren Diakinese fanden wir bei

reinen Rassen die aus Chromatiden gebildeten Ringtetraden, die *Philosamia cynthia* besonders schön lieferte. An Ringtetraden waren 13 bei *Walker*i und 14 bei *Pryer*i vorhanden, von denen die einen aus einem Chromosomennukleolus hervorgebracht wurden, wie DEDERER schon berichtete. Die Kontraktion bezog sich auf alle Tetraden bei *Walker*i und *Pryer*i fast gleichartig, um endlich in der Prophase in

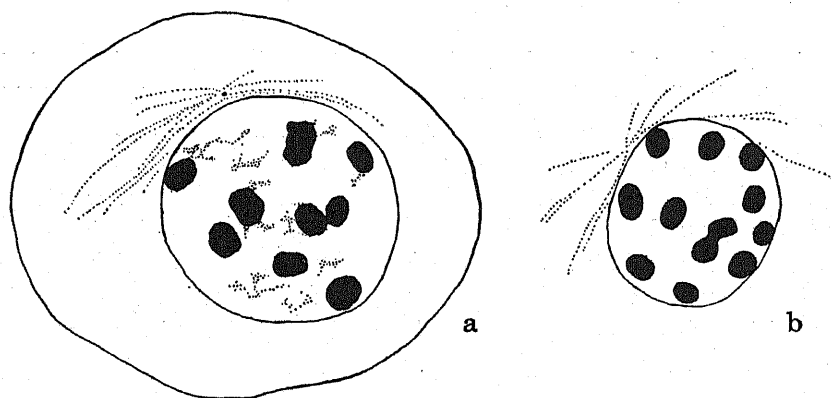


Abb. 5. Zwei Kerne in Diakinese bei *Pryer*i. Vergrößerung 2400 mal. b ist in etwas späterem Stadium als a.

semmel-förmiger Gestalt zu erscheinen. In dem kurzen Stadium der späteren Diakinese tritt aber eine merkwürdige Figur in den Chromosomen nur bei *Walker*i ein, indem zwei von 14 immer sich gegenseitig nähern, während die anderen noch an der Kernmembran von einander getrennt liegen, wie Abb. 5 zeigt. Diese eigenartige Chromosomenassoziation erhielt sich selten bis zur Metaphase (Tafel 1, 9). Die Bedeutung dieser Erscheinung wird später erwähnt werden.

Die normalen Tetraden der  $F_1$ -Bastarde verhielten sich

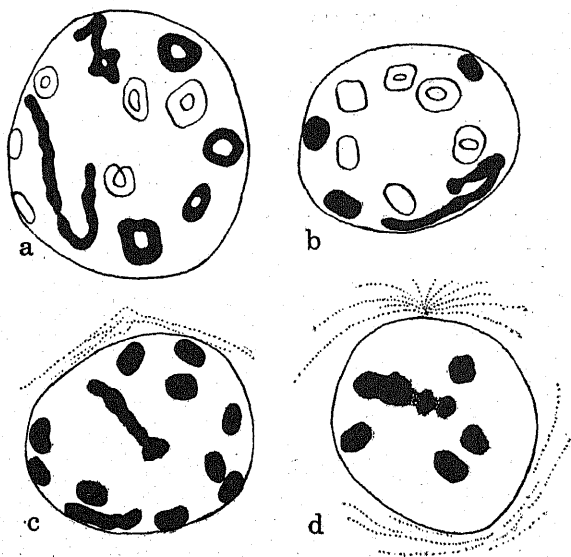


Abb. 6. Vier Kerne von der Diakinese bis zur Prophase beim  $F_1$ -Bastard. Vergrößerung 2400 mal. a, b. Diakinese. c. Spätere Diakinese. d. Prophase.

genau so wie die der *Pryeri*, aber zwei abnorme Tetraden ganz anders. Die eine davon, die wahrscheinlich später zum dreiteiligen Element werden würde, bestand aus einer normalen Ring-Tetrade und einem kürzeren oder längeren Stab, der mit dem einen Ende an der Verbindungsstelle der den Ring bildenden zwei Chromosomen anhaftete. Sie war also ein Trisom. Solche Chromosomenkette fanden wir ganz deutlich oben in der Abb. 6, a und in der Taf. 38, 8 (aus demselben Kern).

Die andere wies aber kein ringförmiges, sondern sehr lange, bald bogenförmig, bald schlangenförmig gekrümmte Elemente auf (Abb. 6). Es scheint mir, als ob sie aus zwei Chromosomenfäden, die sich miteinander am Ende kettenweise verklebt hatten, entstanden sei. In Abb. 6, a kann man eine Einschnürung, die wohl die Stelle der Verkettung zeigt, an dem langen Chromosomenfaden, der links innen im Kern liegt, beobachten. Jeder der beiden Teile wurde auch wohl aus einer Kette von je zwei Chromosomen gebildet. Je weiter deshalb die Kontraktion der Chromosomen fortschreitet, desto deutlicher werden die vier Gliederungen (Abb. 6, d).

#### IV. Deutung der Chromosomenverkettung und der Chromosomenverhältnisse der *Walkeri* und *Pryeri*

In den Stadien von der Diakinese bis zur Metaphase der ersten Reifeteilung beim  $F_1$ -Bastard, wurden, wie ich oben erwähnt habe, die zwei Arten der Verkettung in einer und derselben Zelle beobachtet, und zwar eine Kette von drei Chromosomen, ein Univalent und ein Bivalent, und die andere von vier univalenten Chromosomen. Selbstverständlich wurde keine von diesen in reinen Rassen angetroffen, sondern nur durch die Bastardierung hervorgebracht. Die Erklärung kann man wahrscheinlich in der äußeren und inneren Veränderung der Chromosomen einer Rasse, die phylogenetisch neu ist, suchen.

Wenn auch die Bestimmung des phylogenetischen Alters dieser beiden Rassen sehr große Schwierigkeit bietet, können wir doch als höchst wahrscheinlich annehmen, daß die chinesische viel älter als die japanische ist. *Philosamia cynthia* hat vermutlich ihre Ursprungsheimat in Indien oder Süd-China und verbreitete sich von dort nach Japan.

So kann wohl gesagt werden, daß *Walkeri* mit 13 Chromosomen die primäre, *Pryeri* mit 14 Chromosomen dagegen die sekundäre Rasse ist. Eine Spur der Verringerung der Chromosomenzahl bei *Walkeri* findet man nicht.

Wenn die Vermutung also richtig ist, daß die Chromosomenzahl sich vergrößert habe, so würden zwei Möglichkeiten für die Entstehung der Vermehrung der Zahlen anzunehmen sein: nämlich die



eine durch Fragmentation eines Chromosoms und die andere durch Verdoppelung eines Chromosoms.

Bevor wir auf diese Möglichkeiten weiter eingehen, wollen wir die Chromosomenkette von drei Chromosomen im  $F_1$ -Bastarde betrachten. Die Konfiguration der Kette in der Diakinese zeigt ein typisches Bild von Trivalent.

Also besteht die Deutung dieser Kette eher in der Annahme, daß ein Chromosom der Grundzahl 13 von *Pryeri* durch einen besonderen zytologischen Vorgang verdoppelt wurde, als in der des Zerfalls eines Chromosoms. Die verdoppelten Chromosomen waren durch die Veränderung ihrer inneren Struktur nach verschiedenen Richtungen mehr oder weniger so modifiziert, daß sie bei *Pryeri* nicht mehr zu konjugieren imstande zu sein scheinen. Gestützt wird diese Auffassung vor allem durch das Verhalten von zwei Chromosomen nur bei *Pryeri* im Stadium der späteren Diakinese. Wie wir schon zeigten, findet eine sekundäre Assoziation in diesem Stadium, selten sogar in der Metaphase der Reifeteilung statt. Das bedeutet, daß diese zwei Chromosomen verwandt sind.

Dann sollte man beim Bastarde erwarten, daß die zwei phylogenetisch verwandten *Pryeri*-Chromosomen mit den homologen Teilen des *Walkeri*-Chromosoms konjugieren und sich infolgedessen eine Verkettung von drei Chromosomen im  $F_1$ -Bastarde zeigen werde.

Bei der langen Chromosomenkette, die wahrscheinlich das Fehlen eines Chromosoms im  $F_1$ -Bastarde verursacht, drängt sich die Vermutung auf, daß ein Chromosomenstück bei der sekundären Rasse *Pryeri* sich durch Translokation an ein anderes nicht homologes Chromosom angeheftet habe und dadurch die fast unmerkliche Veränderung des Karyotypus bei *Pryeri* hervorgerufen worden sei.

Da der verlagerte Chromosomenteil von *Pryeri* mit dem entsprechenden Teile von *Walkeri* bei  $F_1$ -Bastard konjugiert, findet man ein langes Sammelchromosom, das aus vier an den Enden verklebten, nicht homologen Chromosomen entstanden ist.

Das Vorkommen eines Rings oder, bei Öffnung einer Stelle desselben, einer Kette von vier Chromosomen (sogen. Amphibivalent nach HÅKANSSON 1931) wurde schon bei einigen Pflanzen-Bastarden gezeigt, nämlich bei *Pisum* (HÅKANSSON, 1929, 1931, 1932), bei *Datura* (BELLING und BLAKESLEE, 1924, 1926), bei *Polemonium* (COOPER und BRINK, 1931; CLAUSEN, 1932) und bei *Zea Mays* (McCLINTOCK, 1930; BURNHAM, 1930, 1932; BRINK und COOPER, 1932). Bei allen Fällen finden die Autoren ihre Erklärung darin, daß eine einfache Translokation von einem Chromosomenende zum Ende eines nicht homologen oder der Austausch zwischen nicht-homologen stattgefunden habe. Die Fälle der Chromosomenkette



wurden meistens durch einfache Translokation erklärt. Jedenfalls hat BURNHAM bei *Zea Mays* darauf hingewiesen, daß die Kette auch beim Austausch stattfinden konnte, wobei die Austauschstücke kurz waren.

Meistens zeichneten sich die Pflanzen mit Chromosomenverkettung durch Gonensterilität aus. Diese beruht auf der Chromosomenverteilung, wobei ein Stück fehlt, ohne daß aus einem verlagerten Stück ein Ersatz auf die Gonen wirkt.

Aber bei *Philosamia cynthia* habe ich die Sterilitätserscheinungen leider noch nicht untersucht. In der Seitenansicht der ersten Metaphase sieht man verschiedene Gestalten in der Anordnung der Chromosomenketten, durch die vielleicht ein gewisser Grad von Spermien-Sterilität verursacht werden könnte.

Allerdings ist es wohl kaum zu kühn, zu vermuten, daß im Laufe der phylogenetischen Entwicklung der kontinentalen 13-Chromosomenrasse zur japanischen 14-Chromosomenrasse zufällig zwei Erscheinungen zusammenfallen: die Verdoppelung eines Chromosomenpaares einerseits und die Translokation eines Chromosomenstückes zu einem anderen nichthomologen andererseits.

Somit ist es höchst wahrscheinlich, dass die Prozesse der Translokation und Verdoppelung, außer der Verschmelzung und dem Zerfall der Chromosomen, auf die Phylogenie der Chromosomen bei den heutigen Schmetterlingen einen großen Einfluß ausgeübt haben.

Schließlich wollen wir auf die Frage der Schwankung der Chromosomenzahl bei *Philosamia cynthia*, die von DEDERER beobachtet wurde, zurückkommen. Höchst wahrscheinlich sind einige Reihen in ihren Kulturen, die die Chromosomenzahl 12 zeigen, dem  $F_1$ -Bastarde zwischen *Walkeri* und *Pryeri* oder einem ihrer Nachkommen zuzuschreiben. Weiter kann man vermuten, daß die Individuen, die 13 und 14 Chromosomen in einem und demselben Hoden besitzen, auch zu solchen Nachkommen gehören. Jedenfalls ist es zu bedauern, daß die karyologische Untersuchung der Nachkommenschaft von Bastarden der beiden Rassen bei uns noch nicht vollständig ist. Sie läßt also weitere vollständigere Ergebnisse als erwünscht erscheinen, weil der hier beschriebene Fall sich bei weiterer Untersuchung als höchst interessant erweisen kann.

### Zusammenfassung

1) *Walkeri*, eine kontinentale Rasse von *Philosamia cynthia* DRURY, die phylogenetisch wahrscheinlich primär ist, hat 13 haploide Chromosomen, wie schon DEDERER festgestellt hat.

2) *Pryeri*, die japanische Rasse davon, hat dagegen 14 haploide Chromosomen.

3) In der späteren Diakinese während der Spermatogenese hat nur bei den letzteren immer eine Assoziation zwischen einem Paar Tetraden stattgefunden. Selten bleibt sie bis zu Metaphase in der ersten Teilung.

4) Beim  $F_1$ -Bastard zwischen beiden Rassen fand man nur 12 haploide Chromosomen, worunter zwei merkwürdige Chromosomenverkettungen beobachtet wurden.

5) Die eine ist aus einem normalen Bivalent und einem Univalent entstanden. Diese bilden in Diakinese einen Ring und einen Stab, der an einem Ende mit dem Ring verbunden ist. Das ist also ein typisches Bild von Trivalent in der Diakinese.

6) Unter Berücksichtigung der sekundären Assoziation von einem Paar Tetraden bei der 14-Chromosomen-Rasse, *Pryeri*, und des Bildes von Trivalent in der Diakinese wurde als höchst wahrscheinlich angenommen, daß eins von den 13 Chromosomen durch einen besonderen zytologischen Vorgang verdoppelt worden sei und nachher nach verschiedenen Richtungen innere Veränderungen erfahren habe. Diese verdoppelten Chromosomenpaare konjugieren deshalb bei *Pryeri* nicht mehr miteinander.

7) Erst durch Bastardierung konjugieren diese zwei verwandten Chromosomen in *Pryeri* mit dem ursprünglichen Chromosom in *Walkeri*.

B) Betreffs der Kette von vier univalenten Chromosomen finden wir ganz ähnliche Beispiele bei Pflanzen-Bastarden, nämlich bei *Pisum*, *Datura*, *Polemonium* und *Zea Mays*. Die für diese von den betreffenden Autoren angegebene einfache Erklärung kann man auch für unsere Fälle bei Schmetterlingen anwenden: Es hat nämlich Translokation eines Chromosoms an einem Ende stattgefunden.

9) Die Chromosomenaberrationen von *Philosamia cynthia*, die DEDERER in ihren Kulturen im Laboratorium fand, sind wahrscheinlich den Nachkommen der Bastarde zwischen den 13- und 14-Chromosomen-Rassen zuzuschreiben.

#### Literatur

- Belling, J. and Blakeslee, A. F. 1924. The configurations and sizes of the chromosomes in the trivalents of 25-chromosome *Daturas*. Proc. Nat. Acad. Sci. 10.  
 — and — 1926. On the attachment of non-homologous chromosomes at the reduction division in certain 25-chromosome *Daturas*. Proc. Nat. Acad. Sci. 12.  
 Brink, R. A. and Cooper, D. C. 1932. A structural change in the chromosomes of maize leading to chain formation. Amer. Nat. 66.  
 Burnham, C. R. 1930. Genetical and cytological studies of semisterility and related phenomena in maize. Proc. Nat. Acad. Sci. 16.  
 — 1932. An interchange in maize giving low sterility and chain configurations. Proc. Nat. Acad. Sci. 18.  
 Clausen, J. 1932. Genetic studies in *Polemonium*. III. Preliminary account on the cytology of species and specific hybrids. Hereditas 16.

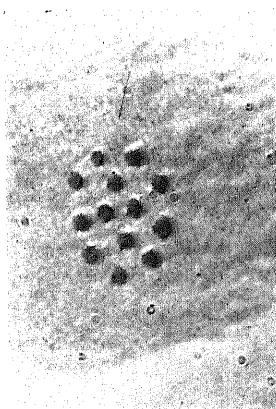
- Cooper, D. C. and Brink, R. A. 1931. Cytological evidence for segmental interchange between non-homologous chromosomes in maize. *Proc. Nat. Acad. Sci.* 17.
- Dederer, P. H. 1907. Spermatogenesis in *Philosamia cynthia*. *Biol. Bull.* 13.
- 1915. Oogenesis in *Philosamia cynthia*. *Journ. Morph.* 26.
- 1928. Variations in chromosome number in the spermatogenesis of *Philosamia cynthia*. *Journ. Morph.* 45.
- Gairdner, A. E. and Darlington, C. D. 1931. Ring formation in diploid and polyploid *Campanula persicifolia*. *Genetica* 13.
- Håkansson, A. 1929. Chromosomenringe in *Pisum* und ihre mutmaßliche genetische Bedeutung. *Hereditas* 12.
- 1931. Über Chromosomenverkettung in *Pisum*. *Hereditas* 15.
- 1932. Neue Fälle von Chromosomenverkettung in *Pisum*. *Hereditas* 16.
- Hammerland, C. and Håkansson, A. 1930. Parallelism of chromosome ring formation, sterility and linkage in *Pisum*. *Hereditas* 14.
- Kawaguchi, E. 1928. Zytologische Untersuchungen am Seidenspinner und seinen Verwandten. I. Gametogenese von *Bombyx mori* L. und *Bombyx mandarina* M. und ihrer Bastarde. *Zeits. Zellforsch. mikr. Anat.* 7.
- Pellew, C. and Sansome, E. R. 1931. Genetical and cytological studies on the relation between Asiatic and European varieties of *Pisum sativum*. *J. Genet.* 25.
- Sansome, E. R. 1932. Segmental interchange in *Pisum sativum*. *Cytologia* 3.

#### Figurenerklärung zu Tafel 38

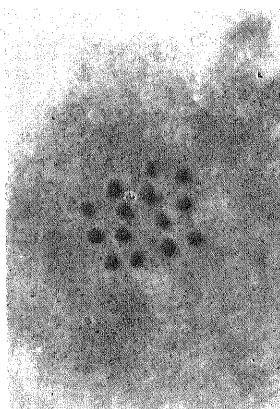
Mikrophotographien 1-7, 9 wurden mit Hilfe von LEITZ „Makam“ unter Benutzung von ZEISS hom. imm. Obj. 1.5 mm. Apert. 0.58 und einem LEITZ periplanen Okular 15×, Photo. 8 einem peripl. Ok. 8× aufgenommen. Vergrößerung etwa 1800 mal in Photo. 1-7, 9, etwa 960 mal in Photo. 8. Sämtliche Photographien sind unretuschiert.

Abbildungen 1, 3, 9 sind nach gequetschten Präparaten von Hoden ausgeführt, die mit Carminessigsäure fixiert und gleichzeitig gefärbt wurden. Abb. 2, 4-8 sind nach Schnittpräparaten aufgenommen, die in CARNOYS Flüssigkeit fixiert und mit HEIDENHAIN'S Eisenhämatoxylin gefärbt wurden.

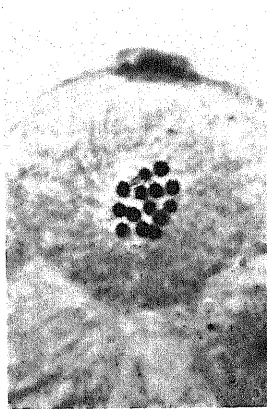
- 1, 2. *Philosamia cynthia Walkeri*. Erste Reifeteilung.
- 3, 4, 9. *Philosamia cynthia Pryeri*. Erste Reifeteilung.
5. *Ph. c. Walkeri*. Zweite Teilung.
- 6, 7. *Walkeri* × *Pryeri*. Äquatorialplatten mit zwei Chromosomenverkettungen. Oben im Bild von 7 sind zwei von den 12 Chromosomen in einen anderen Schnitt gefallen und deshalb hier unsichtbar.
8. Links zeigt sich ein Kern in Diakinese mit zwei Ketten und einigen Ring-tetraden. Die Zeichnung aus demselben Kern steht in Abb. 6, a.



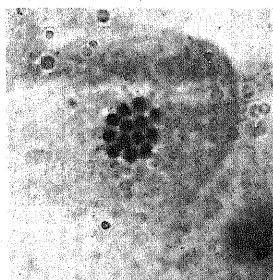
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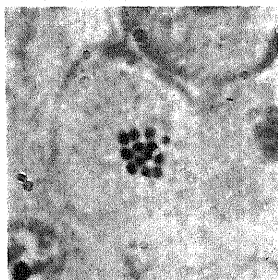
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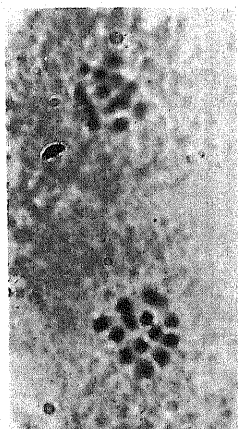
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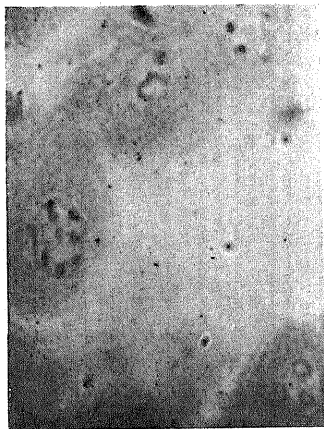
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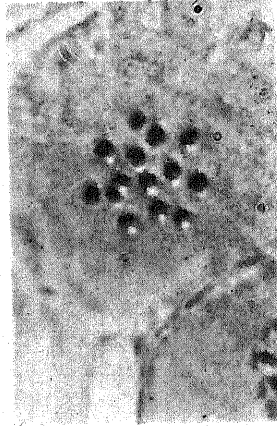
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9

Kawaguchi: Chromosomenketten beim Rassenbastarde des Seidenspinners  
*Philosamia cynthia*.



## On the Fertilization of *Nelumbo nucifera*

By

Ichiro Ohga

(With One Plate)

Early in 1904 York studied the American species, *Nelumbo lutea* and described its fertilization, floral development and embryo formation. While studying the Japanese species, *N. nucifera* we have had an opportunity to investigate the pollination and fertilization of this plant for the past few years. In the present paper some results of the experimental and cytological observations carried out during last summer will be presented.

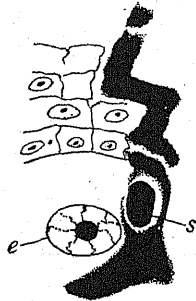
Material was collected from a Chinese variety grown and cultivated in the lotus field at Kisarazu, Tiba-prefecture. The ovaries at various developmental stages, their lower half being removed, were fixed at intervals of half an hour from 6 a.m. to 6 p.m., August 20 to 23, 1936, in either Bouin's fixative or Navashin's solution. The latter gave better results than the former. The material was passed through the alcohols as usual and imbedded in paraffin. Sections cut from 10 to 15  $\mu$  in thickness were stained with Heidenhain's iron-alum haematoxylin.

On the first day of flowering and on the morning of the second day, the embryo-sac already comes to maturity, in which state an egg and one fused or two as yet unfused polar nuclei are noticeable in their fine spireme stages (Plate 39, Figs. 1-4). The egg is spherical and near its central region one nucleus is located (Plate 39, Fig. 4). The synergids are found almost always to have degenerated. The polar nuclei are situated a short distance below the egg cell. In some embryo-sacs the two polar nuclei are still a little apart; while in other cases they are beginning to fuse. Most commonly these nuclei are found in close contact with each other, though in some cases they have already partially fused (Plate 39, Figs. 1-4).

On the early morning of the second flowering day, pollen grains reach the stigma by the aid of small insects and there begin to germinate. Only a few of the pollen tubes thus developed are able to attain the ovary through a short style having a length of about 1 mm. The pollen tube enters the embryo-sac directly through the micropyle and penetrates five or six layers of the nucellus tissue, the apex attacking the lateral side of the egg (Text-fig. 1).

It was proved in the present case of *N. nucifera* that the pollen tube penetrates the style and reaches the embryo-sac during a period

between about 6-8 hours after pollination occurred at about 6 to 7 a.m. on the second day of flowering; and that the fertilization follows soon after. In Text-fig. 2 two densely stained male nuclei which became free from the pollen tube are found in the micropylar



Text-fig. 1. Pollen tube attacking the egg. e, egg nucleus; s, sperm nucleus.  $\times 1300$ .

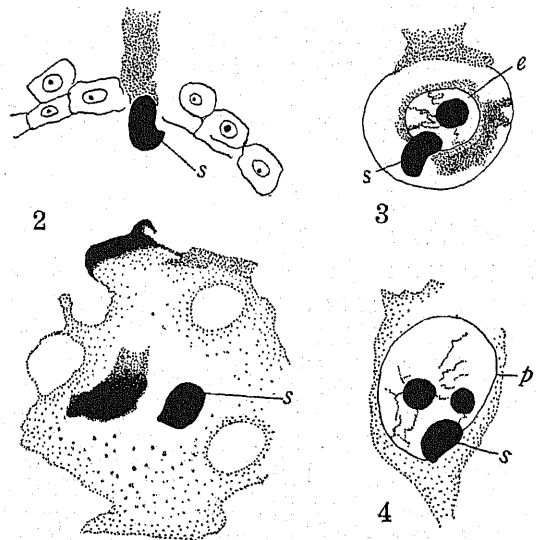
portion of the embryo-sac. They are smaller than both egg nucleus and polar nuclei and retain their ellipsoidal form during fusion (Text-figs. 3, 4). One of the male nuclei fuses with the large polar-fusion nucleus (Text-fig. 4), while the other fuses with the egg nucleus at about the same time (Text-fig. 3).

The fusion of the three nuclei is not carried out in a uniform way in different plants or even in the same plant under varying conditions. A male nucleus and two polars fuse simultaneously in *Potamogeton lucens* (Cook, 1908) and in *Nelumbo lutea* (York, 1904), while in *Castalia odorata* (Cook, 1902) and *Nymphaea advena* (Cook, 1902, Seaton, 1908) the second male nucleus is added to the polar-fusion nucleus as in the present case of *N. nucifera*.

The primary endosperm nucleus undergoes division immediately after its formation (Plate 39, Figs. 5, 6). The axes of the first and second cleavage figures of the fertilized egg are nearly perpendicular to the transverse axis of the embryo-sac (Plate 39, Figs. 7, 8) resulting in a linear three-celled embryo, as described by Cook (1902) in *Castalia* and *Nymphaea*.

In concluding, the pollination in *N. nucifera* occurs with the aid of small insects

about one or two hours after the opening of the flower which



Text-figs. 2-4. 2, micropylar portion of the embryo-sac, a pollen tube entering. 3, fusion of female and male nuclei. 4, fusion of a sperm nucleus with a large polar-fusion nucleus (p). e, egg nucleus. s, sperm nucleus.  $\times 2000$ .





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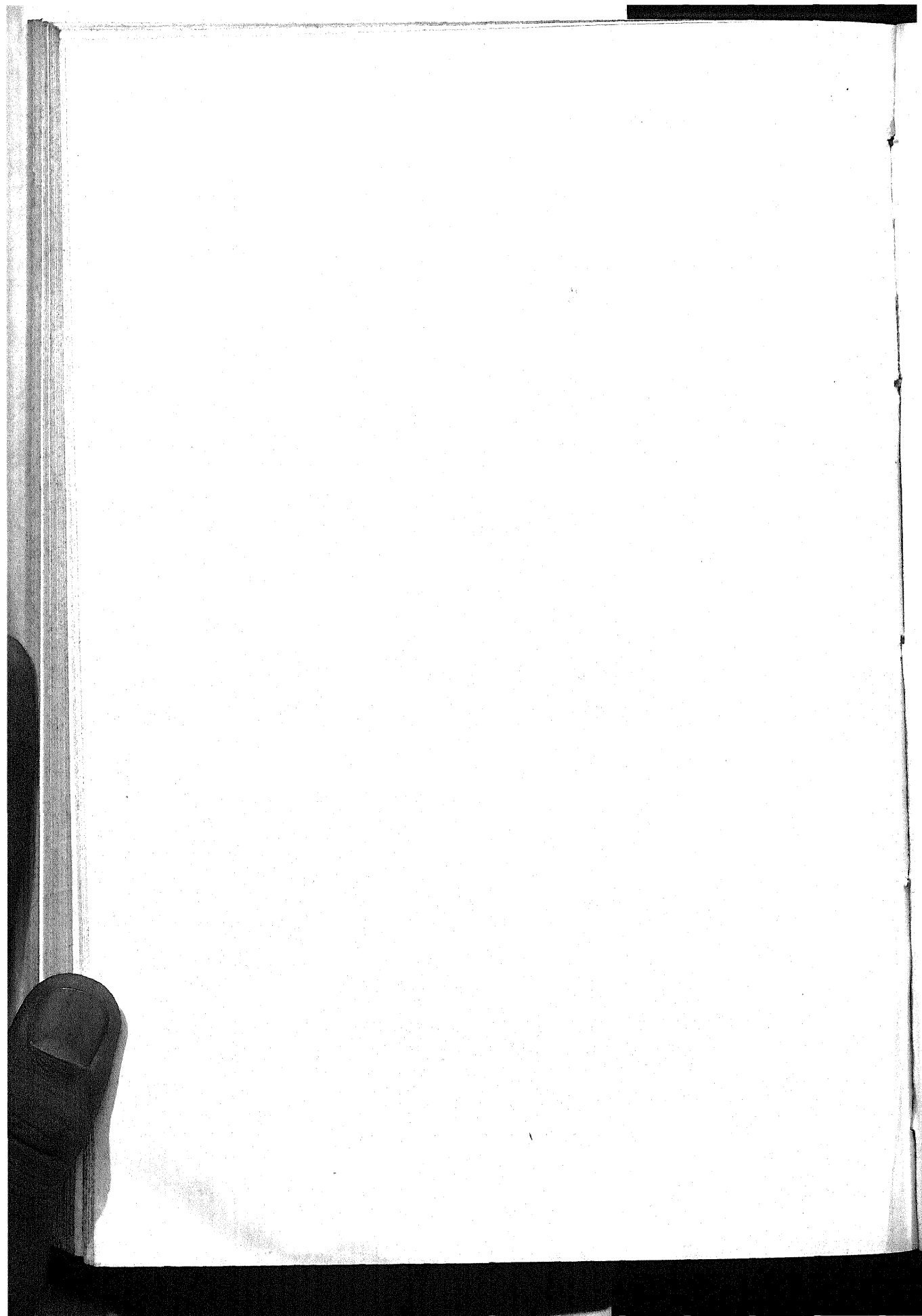


8



9

Ohga: On the Fertilization of *Nelumbo nucifera*



takes place usually at about 5 a.m. on the second day of flowering. Fertilization is accomplished within about 6 to 8 hours after pollination and is followed immediately by division of the primary endosperm nucleus. According to Schnarp's compilation (1929), *Phaseolus vulgaris* and *Secale cereale* are good examples in showing the shortest time intervals from pollination to fertilization; in the former 8-9 hours (Weinstein, 1922) and in the latter 7 hours (Jost, 1907), both obtained under green house conditions. The present case of *N. nucifera* seems to add one more example to these, which example however has been obtained under natural conditions.

The writer wishes to thank Mr. Kazuo Suzuki of the Botanical Institute, Faculty of Agriculture, Tokyo Imperial University, who so gladly offered assistance in preparing materials and photomicrographs.

Botanical Institute, Faculty of  
Agriculture, Tokyo Imp. University

#### Literatures Cited

- Cook, M. T. 1902. Development of the embryo-sac and embryos of *Castalia odorata* and *Nymphaea advena*. Bull. Torr. Bot. Club, 29: 211-220.  
— 1908. The development of the embryo-sac and embryo of *Potamogeton lutens*. ib., 35: 209-218.  
Jost, L. 1907. Über die Selbststerilität einiger Blüten. Bot. Zeit., 65: 77-116.  
Schnarf, K. 1929. Embryologie der Angiospermen. Berlin.  
Schürhoff, P. N. 1926. Die Zytologie der Blütenpflanzen. Stuttgart.  
Seaton, S. 1908. The development of the embryo-sac of *Nymphaea advena*. Bull. Torr. bot. Club, 35: 283-290.  
Weinstein, A. I. 1926. Cytological studies on *Phaseolus vulgaris*. Amer. Journ. Bot. 13: 248-263.  
York, H. H. 1904. The embryo-sac and embryo of *Nelumbo*. Ohio Naturalist, 4: 167-176.

#### Explanation of Plate 39

All photomicrographs are made at a magnification of 2100 diameters except Fig. 9 ( $\times$  ca. 210) and reduced 2/3 in reproduction.

- Figs. 1-3. Successive stages of fusion of two polar nuclei.  
Fig. 4. Micropylar end of a matured embryo-sac showing an egg (upper) and a polar-fusion nucleus (lower).  
Fig. 5. An egg and a primary endosperm nucleus after double fertilization accomplished.  
Fig. 6. Anaphase of the first mitosis of the endosperm nucleus.  
Fig. 7. Telophase of the same. Two celled embryo formed.  
Fig. 8. Partition wall forming between two daughter embryo-sac nuclei.  
Fig. 9. Showing an embryo of two cell stage and two celled embryo-sac.

**Untersuchungen über die Wirkung des elektrischen Stromes auf lebende Zellen. I. Das Verhalten der mitotischen Figur unter der Wirkung des Gleichstromes<sup>1)</sup>**

Von

**Noburô Kamiya**

Kaiserliche Universität Tokyo

(Mit 1 Textfigur)

Was die Wirkung des elektrischen Stromes auf die mitotischen Figuren anbelangt, wiesen PENTIMALLI (1909, 1912), McCLENDON (1910), WAKAYAMA,<sup>2)</sup> BOTTA (1932) u.a. darauf hin, daß der Gleichstrom beim Durchgang durch die sich teilende Zelle intrazellulär eine anodische Verschiebung der Chromosomen hervorruft; deshalb kamen sie zum Schluß, daß die Bewegung der Chromosomen für ihre Elektrophorese gilt. Die Untersuchungsergebnisse hierüber waren jedoch nicht immer endgültig, und zwar lehnten HARDY (1913), MEIER (1921), ZEIDLER (1925) u.a. die Verlagerung der Teilungsfigur ab. Diese sich widersprechenden Ergebnisse erhielt man, ausgenommen die in vitro kultivierten Hühnerembryoherzen von BOTTA, meistens bei den Keimwurzeln von verschiedenen Pflanzen wie *Hyacinthus*, *Allium*, *Pisum*, *Vicia*, usw., wobei die obigen Autoren die Wirkung des Gleichstromes bei den Fixierpräparaten feststellten. Es ist selbstverständlich, daß die Fixierpräparate bei solchen Experimenten nicht immer das reale Verhalten des im lebenden Zustande sich befindlichen Zellinnern zum Ausdruck bringen.

Daher bemühte ich mich in der vorliegenden Arbeit das Verhalten der mitotischen Figur, im lebenden Zustand der Zelle mit besonderer Rücksicht auf ihre Verschiebbarkeit unter der genannten Einwirkung zu verfolgen. Die Untersuchungsergebnisse sind hier je nach der Intensität des Stromes und der Wirkungszeitdauer in Versuch 1 und 2 zusammengestellt.

**Material und Methode**

Als Untersuchungsmaterial wurden junge Staubfadenhaarzellen von *Tradescantia reflexa* verwendet, welche sich in Knospen in ca. 4–5 mm Länge befinden.

Um beim Experimente die konstante Konzentration des Mediums beibehalten zu können, fügte ich der Feuchtkammer ein zweckmäßig

1) Contributions from the Divisions of Plant-Morphology and of Genetics, Botanical Institute, Faculty of Science, Tokyo Imperial University, No. 188.

2) Zitiert aus der Monographie von FUJII (1931).

hergestelltes Kanälchen hinzu, in das das Material mit dem umgebenden Medium eingebettet wird. Ich fertigte mir dieses einfach aus zwei in mäßigem Abstand (5 mm) und parallel gelegten Glasstäbchen in einem Durchmesser von ca.  $80 \mu$  und einem Deckglasstückchen in Breite von ca. 6 mm an. Der Querschnitt des Kanälchens beträgt danach also ungefähr  $0,5 \text{ mm}^2$ . Dann brachte ich mit beiden Öffnungen des Kanälchens ein Bröckchen von 2% Agar-Agar in Berührung, wobei das Agar die gleiche Lösung wie das, das Kanälchen füllende Medium enthält. Als Medium dienten meistens 0,1 N KCl- und 2% Rohrzuckerlösung. Man führt ein Elektrodenende mittels des Mikromanipulators in beide Agarstückchen hinein, um dadurch den elektrischen Strom möglichst gleichmäßig durch das Kanälchen leiten zu können. Die Stromintensität, welche in den folgenden Versuchen gezeigt wird, entspricht derjenigen, die durch das Medium im Kanälchen leitet, und nicht derjenigen, die wirklich durch die Zelle geht. Als Elektroden dienten die für diesen Zweck geeignet, besonders hergestellten unpolarisierbaren 0,1 N KCl Agar-Elektroden (ca. 1 mm im inneren Durchmesser am vorderen Teil). Da diese eine große Resistanz (über  $50,000 \Omega$ ) haben, erfordert die Stromquelle eine hohe Spannung; dazu dienten erstens Trockenbatterien, maximale Spannung an den Klemmen 135 V, und zweitens, falls es nötig ist, ein selbst hergestellter kleiner Quecksilber-Gleichrichter, Spannung an den Klemmen ca. 660 V. Die Stromstärke im Kanälchen wurde mit Hilfe der zwei Potentiometer geregelt. Die Beobachtungen wurden mittels LEITZ Öl-Immersion  $100\times$  und ZEISS K  $15\times$  ausgeführt.

### **Versuch 1: Kurzdauernde Wirkung des starken elektrischen Stromes**

Die zu diesem Versuche angewendete Stromstärke betrug ca. zwischen 0,2–1,0 mA, wobei die Wirkungsdauer auf 0,5–5,0 Sek. begrenzt wurde.

Wird ein starker Strom durch die Zelle in der Teilung parallel ihrer Längachse entlang geleitet, so bemerkt man, daß die Spindelfigur sich als Ganzes nach der Anodenseite der Zelle verschiebt (Fig. 1). Bemerkenswert ist dabei, daß die einzelnen Chromosomen, solange die Zelle durch den Strom noch unbeschädigt bleibt, ihre Lage im Spindelraum fast gar nicht verändern; ja, es findet fast keine relative Bewegung zwischen den einzelnen Chromosomen und der die Chromosomen umgebenden Spindelsubstanz, d.h. dem Atraktoplasma (FUJII 1931)<sup>1)</sup> statt. Unter der rechtwinkligen Durchströ-

1) FUJII hat in seiner Monographie vorgeschlagen, die den Spindelraum füllende Substanz, in ihrer physikalisch-chemischen Beschaffenheit vom Zytoplasma unterscheidend, das „Atraktoplasma“ zu nennen.

mung gegen die Teilungsachse bewegen oder biegen sich die Chromosomen innerhalb des Spindelraums auch kaum und die Spindel als Ganzes fast nicht. Der Raummangel an der Anodenseite der Zelle

würde hierbei für das Nichtbewegen der Spindelfigur verantwortlich sein.

Falls die Stromintensität verhältnismäßig groß ist, kommt mitunter eine der Plasmolyse ähnliche Erscheinung an der Kathodenseite der Zelle zum Vorschein. Daher geht der Protoplasma wandbelag — wahrscheinlich elektroosmotisch? — an der genannten Seite von der Zellwand ab, wobei die Zelle sich im etwas hypotonisch wirkenden Medium befindet. Mit dem

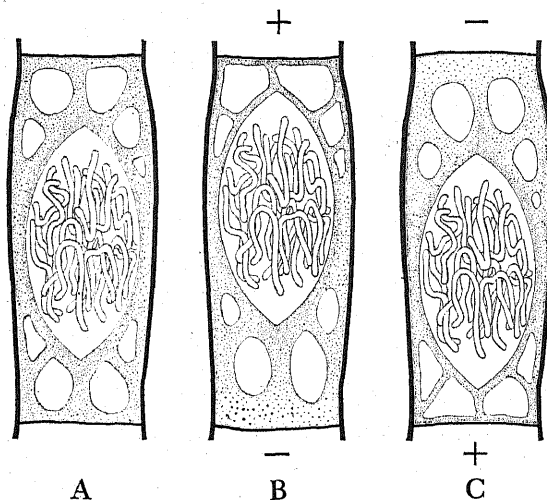


Fig. 1. Eine Haarmittelzelle in der Anaphase, schematisiert. Das angequollene Zytoplasma ist dünn punktiert und das entquollene dicht. A) Vor der Durchströmung. Die Spindel befindet sich in der Mitte der Zelle. B) Durch die Durchströmung bewegt sich die Spindelfigur zur anodischen Seite der Zelle hin. C) Der Pol umgekehrt.

Strom-Abschließen wird aber, wenigstens mikroskopisch, eine augenblickliche Wiederherstellung verbunden.

Leitet man den Strom nicht allzu lange durch die Zelle, und wird die Zelle dadurch nicht so beschädigt, so geht die sich einmal anodisch verlagerte Spindelfigur beim Stromunterbruch fast gleichzeitig schnell zu ihrer Anfangsstelle in der Zelle zurück. Bei der in solcher Weise ein- oder vielmals durchströmten Zelle, wobei die Wirkungsbedingungen eine gewisse Grenze nicht überschreiten, konstatierte ich, daß diese eben nach dem Strom-Unterbrechen ihre Mitose weiter fortsetzt und endlich zwei normale Tochterzellen bildet.

Der intrazelluläre Hin- und Rückgang der Teilungsfigur tritt bei diesen Versuchen erst in der späten Prophase ein und dauert bis zum Beginn der Scheidewandbildung; dieser ist ja in der Meta- und Anaphase am leichtesten.

Beim Übertritt der Stromintensität sowie auch der Wirkungszeitdauer bewegt sich die nach der Anode hin wandernde Spindelfigur infolge der Gelifizierung des Plasmas nicht mehr zurück; das Plasma koaguliert und die Chromosomen werden deutlicher von ihrer



Umgebung unterscheidbar, als ob sie elektrisch fixiert worden sind. Bei der sich in der späten Anaphase befindlichen Zelle übt solcher starke Strom eine bedeutende Verschiebung der mitotischen Figur aus; sie erhält ihre Form nicht mehr und eine Biegung in ihrer Mitte zeigt, worin der Phragmoplast liegt.

Sehr merkwürdig ist das besonders empfindliche Verhalten des Zytoplasmas in der Mitose bei Durchleitung des elektrischen Stromes. In der Tat wird durch die Stromanwendung eine polarisatorische Quell- und Entquellung des Zytoplasmas hervorgerufen, und zwar findet die Entquellung des Zytoplasmas in der Anodenseite der Zelle und gleichzeitig die Quellung desselben in der Kathodenseite statt. Dieses polarisatorische gegenseitige Verhalten des Zytoplasmas in der einzelnen Zelle beobachtete ich in der sich teilenden, besonders der Endzelle des Haares am deutlichsten, da es dort genügende Mengen von Zytoplasma gibt.

### **Versuch 2: Langdauernde Wirkung des schwachen elektrischen Stromes**

Ein schwacher Strom (0,001–0,05 mA) übt in Wirklichkeit morphologisch fast keine sofortige Wirkung auf die Teilungsfigur aus. Jedoch, wenn solcher auf die sich teilende Zelle langdauernd oder immerwährend einwirkt, so tritt eine beträchtliche Hemmung des Teilungsfortganges ein. Wenn eine mitotische Figur in der Prophase mit solchem schwachen Strom fortwährend behandelt wird, so sistiert die Mitose nicht nur ihren weiteren Fortgang, sondern die betroffene Teilungsfigur kehrt zuweilen wieder in den Ruhezustand zurück. Bei der Metakinese sowie auch der Meta- und früheren Anaphase dagegen verzögern sich die Teilungsfortgänge in verschiedenen Graden, sie vollenden jedoch schließlich die Tochterkernbildung.

Der Strom ruft beim Durchgang durch die Zelle keine bedeutende Verschiebung der mitotischen Figur, wie im Versuch 1, hervor, doch entsteht die Scheidewand etwas von der Mitte der Zelle entfernt an einem atypischen Ort. Öfters verschiebt sich die Scheidewandanlage etwas nach der Anode, aber zuweilen etwas nach der Kathode. Aus den Ergebnissen der wiederholten Versuche komme ich zu der Folgerung, daß die Scheidewandbildung unter solcher Bedingung Neigung zeigt, sich nach der Spitze des Haares zu verlagern.

Bei dem Versuch 1 sowie diesem konnte ich jedoch nicht feststellen, ob sich einzelne Chromosomen aus der Spindelfigur loslösen und sich nach der Peripherie der Zelle verschieben, wie die Resultate PENTIMALLI's und auch die BOTTA's ergaben. Bisweilen wurden dennoch nach langer Durchströmung selten beträchtliche Ver-



änderungen in der Anordnung der Chromosomen bemerkt. Diese Veränderungen sind meines Erachtens nicht für eine direkte, sondern vielmehr für eine sekundäre Wirkung des Stromes zu halten; sie treten vermutlich infolge der elektrisch hervorgerufenen Hydrationsveränderung des Zellinnern auf. Nur selten führt die Zelle in der Mitose durch dauernde Anwendung des schwachen Stromes eine frühzeitige Scheidewandbildung herbei; in der Metaphase kommt deshalb schon eine Scheidewandanlage zum Vorschein, welche sich später zu einer solchen unvollendeten entwickelt.

Was das Verhalten des Zytoplasmas bei diesem Versuche betrifft, so erfährt es morphologisch fast keine bedeutende Veränderung, und zwar findet weder die elektrophoretische Verschiebung noch eine auffallende polarisatorische Quell- und Entquellung desselben statt. Wird der Strom immerdauernd schräg gegen die Haarachse geleitet, so findet die Scheidewandbildung auch rechtwinklig gegen dieselbe statt, wobei sie jedoch, wie oben erwähnt wurde, nicht immer in der Zellmitte, sondern manchmal am atypischen Orte liegt.

### Schlußbetrachtung

Aus den Ergebnissen der vorliegenden Versuche (Versuch 1) stellte ich erstens fest, daß unter der Einwirkung des Gleichstromes auf die lebenden Zellen von *Tradescantia*-Haaren keine relative Bewegung zwischen den Chromosomen und der sie umgebenden Spindelsubstanz (Atraktoplasma) eintritt, indem die Spindel als Ganzes mitsamt ihrem Innern nach der Anode fortgeht; und zweitens, wenn die Spindelfigur beim Stromunterbruch sofort zur Anfangsstelle zurückkehrt, so nimmt sie wieder die Lage der Zellmitte ein.

Bekannt ist, daß die elektrophoretische Verschiebungsfähigkeit des Teilchens nur wenig auf seinem Innern, sondern vielmehr auf der Oberflächen-Eigenschaft, nämlich auf dem Zeta-Potential beruht; danach scheint es mir immerhin noch nicht einwandfrei geklärt zu sein, daß die anodische Bewegung der Chromosomengruppe oder der Spindel der Ladung der Chromosomen zugeschoben werden kann. Falls man den Fortgang der mitotischen Figur für ein elektrophoretisches oder elektroosmotisches Verhalten ansieht, bleibt die durch den Stromunterbruch hervorgerufene Erscheinung des sofortigen Rückganges oder der Herbewegung der Spindelfigur unerklärlich, es sei denn, daß man eine starke Elastizität des Protoplasten annimmt.

Das Verhalten der Spindelfigur beim Versuch 1, in dem keine relative Bewegung der Chromosomen gegen die Spindelsubstanz ein-

tritt, überzeugt, daß die durch den elektrischen Strom hervorgerufene Quell- und Entquellbarkeit des Zytoplasmas für die intrazelluläre Verschiebbarkeit der Spindelfigur eine wichtige Rolle spielt.

Es ist eine bekannte Tatsache, daß der Gleichstrom eine Störungswirkung des Ionenkonzentrationsgleichgewichts auf beiden Seiten des Diaphragmas ausübt, und zwar nimmt die H-Ionenkonzentration auf der Anodenseite ab und auf der Kathodenseite zu (vgl. BETHE, A. u. TOROPOFF, T. 1915 a, b). Dieselbe Beziehung kann auch für die Zelle, die mit der Zell- und Plasmamembran verschlossen ist, gefolgert werden, indem das Zytoplasma an der Anodenseite der Zelle (infolge der H-Ionenkonzentrationszunahme) entquillt; und dagegen an der Kathodenseite stark quillt. Unter solchen Umständen ist es begreiflich, daß die Spindelfigur zwischen den beiden Scheidewänden beim Ein- und Abschalten des Stromes wegen der polarisatorischen Hydrationsveränderung des Zytoplasmas lediglich passiv hin- und hergeschoben wird.

Die weitere Bearbeitung über die Stromwirkung sowie das Ladungsproblem des Zell- und Kerninnern bleibt einem zukünftigen Versuche vorbehalten, da ich dafür vielseitiger und ergänzender Untersuchungen bedarf.

Zum Schluß sei es mir gestattet, Herrn Dr. SINOTÔ, unter dessen wohlwollender Leitung die vorliegende Arbeit entstanden ist, an dieser Stelle meinen verbindlichsten Dank auszusprechen. Desgleichen bin ich Herrn Dr. WADA für seine liebenswürdigen Anweisungen bei der Durchführung dieser Untersuchungen zu großem Danke verpflichtet.

### Zusammenfassung

Die Verschiebbarkeit der mitotischen Figur unter der Einwirkung des Gleichstromes wurde bei den Staubfadenhaarzellen von *Tradescantia reflexa* im lebenden Zustande untersucht. Die wichtigen Ergebnisse lauten wie folgt:

1. Die Spindelfigur verlagert sich unter der Einwirkung eines geeignet starken Stromes als Ganzes nach der Anode, wobei eine relative Bewegung zwischen den Chromosomen und der Spindelsubstanz (Atraktoplasma) kaum erkennbar ist; beim Stromunterbruch kehrt sie auch als Ganzes schnell zu ihrer Anfangsstelle zurück und kommt danach wieder in die Mitte der Zelle. Diese reversible Hin- und Herbewegung der Spindelfigur wird daher nicht als Elektrophorese der Chromosomen angesehen; sie scheint vielmehr auf verschiedenen polarisatorischen Veränderungen des Zytoplasmas zu beruhen.

2. Unter dem zu starken Strom oder der zu langen Wirkungs-  
dauer bewegt sich die Spindelfigur einmal auffallend anodisch hin,  
jedoch infolge der Gelifikation des Zytoplasmas kehrt sie nicht mehr  
zu ihrer Anfangsstelle zurück.

3. Die Verschiebbarkeit der Spindelfigur fängt bei der späten  
Prophase an und dauert bis zur Scheidewandbildung, wobei sie in  
der Meta- und Anaphase am stärksten ist.

Genetik-Abteilung, Botanisches Institut,  
Naturwissenschaftliche Fakultät der  
Kaiserlichen Universität zu Tokyo

#### Literaturverzeichnis

- Bersa, E. u. Weber, F. 1922. Reversible Viskositäts-erhöhung des Cytoplasmas unter der Einwirkung des elektrischen Stromes. Ber. Deut. Bot. Ges. 40: 254-258.
- Bethe, A. u. Toropoff, T. 1915a. Über elektrische Vorgänge an Diaphragmen. Teil I. Die Neutralitätsstörung. Zeit. physikal. Chemie 88: 686-742.
- u. — 1915b. Über elektrische Vorgänge an Diaphragmen. Teil II. Die Abhängigkeit der Größe und Richtung der Konzentrationsänderungen und der Wasserbewegung von der H-Ionenkonzentration. Ebenda 89: 597-637.
- Botta, B. 1932. Ricerche sulla carica e sul trasporto elettrico della figura cromatica delle cellule in mitose in culture di cuore embrionale di pollo (Nota Preventiva). Arch. exp. Zellforsch. 12: 455-464.
- Fujii, K. 1931. Zytologie in Vergangenheit und Gegenwart (japanisch). 52 S., Verlag Iwanami. Tokyo.
- Hardy, W. B. 1913. Note on differences in electrical potential within the living cell. Jour. Physiol. 47: 108-111.
- McClendon, J. F. 1910. On the dynamics of cell-division. I. The electric charge on colloids in living cells in the root tips of plants. Arch. f. Entw.-Mech. 31: 80-90.
- Meier, H. F. A. 1921. Effect of direct current on cells of root tip of Canada field pea. Bot. Gaz. 72: 118-138.
- Pentimalli, F. 1909. Influenza della corrente elettrica sulla dinamica del processo cariocinetico. Arch. Entw.-Mech. 28: 260-276.
- 1912. Sulla carica elettrica della sostanza nucleare cromatica. Ebenda 34: 444-451.
- Samejima, R. 1927. Über die durch den elektrischen Strom herbeigeführte Veränderung der Ionen-Konzentration in der Zelle von *Tradescantia virginica* (japanisch). Act. Medi. Hokkaidonensia 5: 435-470.
- Wada, B. 1935. Mikrurgische Untersuchungen lebender Zellen in der Teilung. II. Das Verhalten der Spindelfigur und einige ihrer physikalischen Eigenschaften in den somatischen Zellen. Cytologia 6: 381-406.
- Yamaha, G. 1936. Über die physikalisch-chemischen Untersuchungen der Karyokinese (japanisch). Bot. and Zool. (Tokyo) 4: 305-515.
- Zeidler, J. 1925. Beiträge zur Frage des Galvanotropismus der Wurzeln. Bot. Archiv 9: 157-193.

## Morphology of the Chromosomes of *Drosophila ananassae*

By

**Berwind P. Kaufmann**

University of Alabama

and

Department of Genetics, Carnegie Institution of Washington

### Introduction

*Drosophila ananassae* Doleschall (*D. caribbea* Sturtevant) is widely distributed in tropical regions of both the Eastern and Western Hemispheres (Kikkawa, 1936). Sturtevant (1921) in his study of the North American *Drosophilinae* recorded the occurrence of *D. caribbea* in Brazil, Central America, and the islands of the Caribbean Sea. In the autumn of 1933, and in subsequent years, this species was collected at Tuscaloosa, Alabama, which is considerably north of the southern limit of that area in which freezing temperatures may be expected. The repeated occurrence of the species over the period indicated suggests that it may survive the winter in Alabama, rather than be reintroduced annually. Temporary importation of *D. ananassae* from the tropics to a more temperate region has been reported by Moriwaki (1935), who collected the flies in Tokyo in 1931, but was unsuccessful in subsequent attempts.

The flies breed well in the laboratory under the same culture conditions commonly employed for *D. melanogaster*. Stocks of the Tuscaloosa material which have been maintained over the past three years were used predominantly in the present study, another stock, secured from Japan, serving for comparison.

The chromosomes of *D. caribbea* were first studied by Metz (1916) from material collected in Panama and Cuba. He described the female complement (oogonial) as consisting of four pairs of V-shaped chromosomes, one of which is shorter than the other three. His figures and diagrams of spermatogonial chromosomes show a V-shaped X and a rod-shaped Y. Kaufmann (1936a, 1936b) reported that ganglion cells of the larvae of both the Alabama and the Japanese stocks possess an unequal-armed, J-shaped Y-chromosome. Kikkawa (1936) likewise found a J-shaped Y in spermatogonial cells of his material. In the present article there will be presented a more detailed account of the chromosomes of the ganglia and the salivary glands.

For the study of these cells temporary aceto-carminc preparations were used. Salivary glands remained in the stain about one-half hour, the ganglia for 1-2 hours prior to transferring to a slide. The cells were flattened by rolling a glass vial heavily weighted with mercury across the cover. It was found that if the customary paraffin, gum-mastic seal was supplemented by the occasional application of a thick solution of gum shellac or lacquer, the preparations could be kept in a condition suitable for study over a period of several weeks. Such slides may be made permanent by removing the seal and exposing them to the vapor of dioxan in a closed chamber for a few days.<sup>1)</sup> A thin solution of gum dammar is then permitted to seep beneath the cover. By this method removal of the cover is unnecessary, and the relocation of a given cell in the smear is facilitated; it is especially useful in the study of the neurocytes. Observations and drawings were made using a 1.4 condenser, a 1.4 apochromatic objective, and compensating oculars.

### Chromosomes of the Ganglia

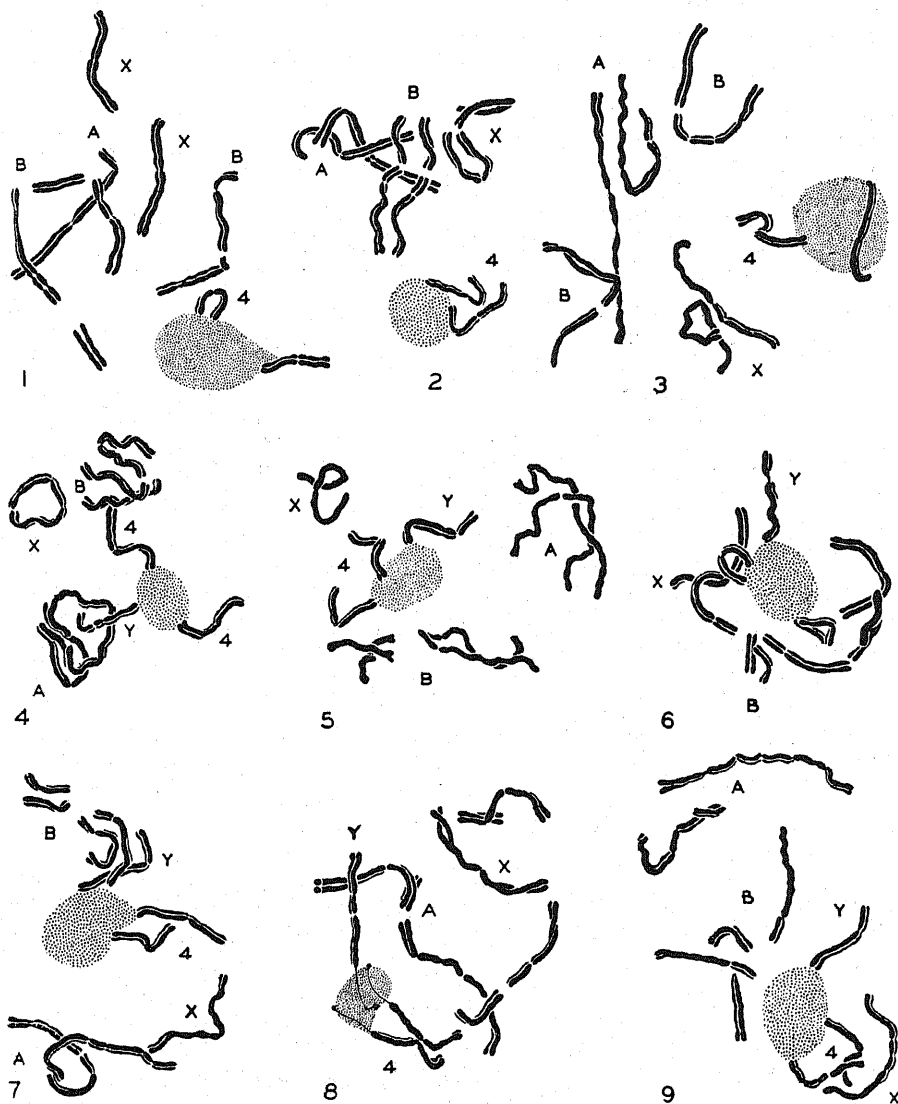
*The Constrictions*—The chromosome complement consists of three pairs of V-shaped autosomes and the sex chromosomes. These are a pair of V-shaped X-chromosomes in the female, an X and a J-shaped Y in the male. The three pairs of autosomes are designated in this paper as A (the longest at metaphase), B (of intermediate size), and 4 (the shortest), since it is uncertain which of pairs A and B correspond with the second and third chromosome linkage groups determined by Moriwaki (1935) and Kikkawa (1936).

The X-chromosomes and all of the autosomes have a median to submedian primary constriction or spindle attachment region (text-figs. 1-22). The secondary constrictions vary considerably in the degree of their visible expression in different nuclei, but a survey of several cells leads to the following interpretation.

Autosomes A in favorable late prophase and early metaphase figures show two distinct secondary constrictions in each arm. Of these the proximal exist at about one fourth of the distance along the longer arm, and one third of the distance along the shorter arm, where they delimit the heteropyknotic areas bordering the primary constriction region. The distal portion of each arm is bisected by another constriction (text-figs. 3, 9). Suggestions of the existence of subterminal constrictions occasionally have been seen (text-figs. 16, 18).

1) The use of dioxan was recommended by Elizabeth G. Lawrence in *Drosophila Information Service* No. 6, 1936.

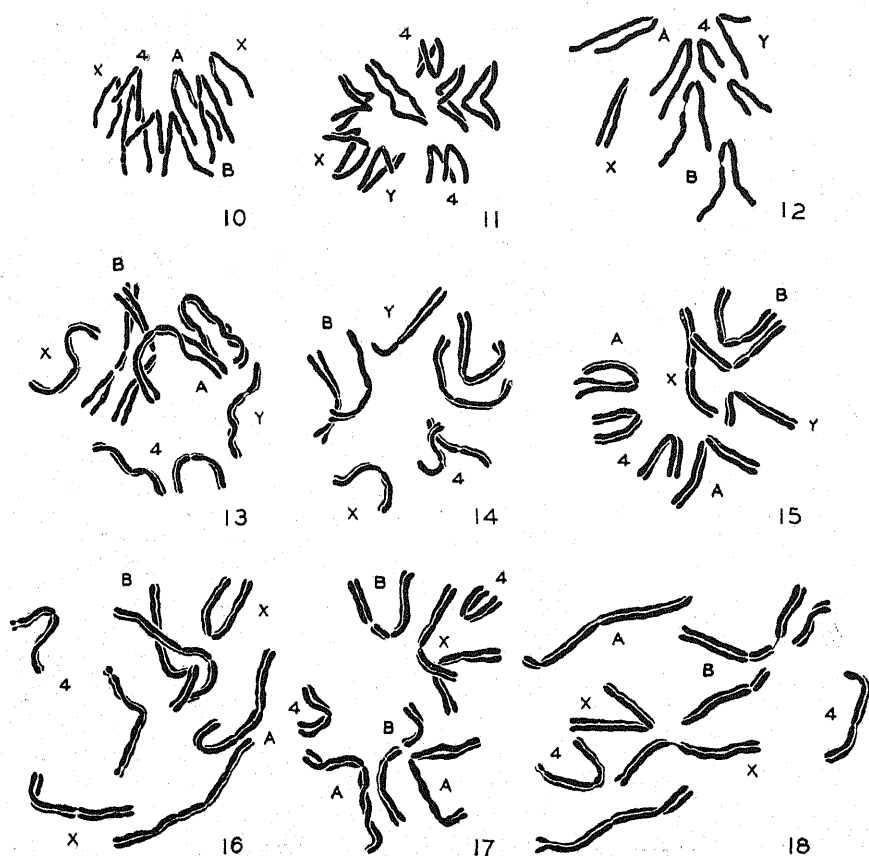
In the B chromosomes the most pronounced constriction is submedian in the longer arm, and during the prophases frequently divides the chromosome into two widely separated portions (see especially text-figs. 5-7). This constriction appears to flank the heteropyknotic area adjacent to the spindle attachment region, as does also a less



Text-Figs. 1-9. Prophases, neurocytes of larvae of *Drosophila ananassae*. 1-3, female, only the fourth chromosomes associated with the nucleolus. 4-9, male, the Y-chromosome also associated with the nucleolus. A—the longest autosomes; B—the second longest autosomes; 4—the shortest autosomes or fourth chromosomes; X—the X-chromosome; Y—the Y-chromosome. Nucleolus stippled.  $\times$  ca. 2900.

pronounced constriction in a similar position in the shorter arm (text-figs. 1, 3).

In the fourth chromosomes both limbs often reveal subterminal constrictions (text-figs. 2, 16, 18). That of the longer limb represents the position at which the development of the nucleolus normally occurs. This may be determined from a consideration of the prophases, when the nucleolus separates a chromomere-like satellite from the remainder of the longer arm. Such detail, which is visible only in deeply stained preparations because of the small size of the satellite, is shown in text-figure 8. The fourth chromosomes alone are associated with the nucleolus in the prophase cells of the female, but in the male three chromosomes maintain such association, since the long arm of the Y also possesses a nucleolus-forming region. As in the autosomes, the satellite is separated from the bulk of the chromosome,



Text-Figs. 10-18. Anaphases (figs. 10-12), late prophase and metaphases (figs. 13-18) of neurocytes of larvae of *D. ananassae*. 10, 16-18 from female larvae; 11-15 from male larvae. Legend as for figs. 1-9.  $\times$  ca. 3500.



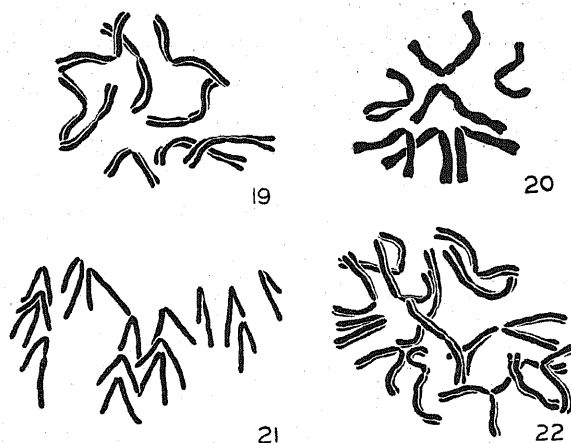
a delicate connecting chromatic thread traversing the nucleolus (text-fig. 8). Another constriction occurs in the long arm of the Y, about one-fourth of the distance from spindle attachment to distal end (text-figs. 4, 7, 8, 13).

In the X-chromosomes, submedian secondary constrictions border the heteropyknotic regions on each side of the spindle attachment region. Other constrictions are subterminal in these chromosomes.

*Heteropyknosis*—In the resting nuclei and in the early prophases, before the chromosome complement becomes defined clearly, certain deeply staining bodies may be recognized. The largest, which lie in contact with the nucleoli, often reveal the tightly coiled chromonemata characteristic of contracted chromosomes. They have been interpreted accordingly as the chromosomes concerned with nucleolus formation, namely, the fourth chromosomes in the female, the fourths plus the Y in the male. Other smaller, deeply staining bodies probably represent those heteropyknotic regions which lie adjacent to the spindle attachment regions in the X and the longer autosomes. Thus, in some male nuclei five pairs of dot-like or rod-like bodies were seen, which may be interpreted as the proximal portions of the chromosomes A, B, and X. At this stage the more distal portions of these chromosomes stain weakly, and are indistinguishable.

*Heteroploid Nuclei*—In several of the ganglia, both male and female, tetraploid cells were observed (text-figs. 21, 22). Usually but one or two such cells per ganglion were seen; occasionally, however, large patches of tetraploid tissue occurred.

Two of the ganglia studied suggested that the individuals from which they were dissected had arisen following non-disjunction. Thus, in one case, all of the cells were trisomic for the fourth chromosome (text-fig. 19); in the other case, only one sex chromosome was present, an XO individual (text-fig. 20).

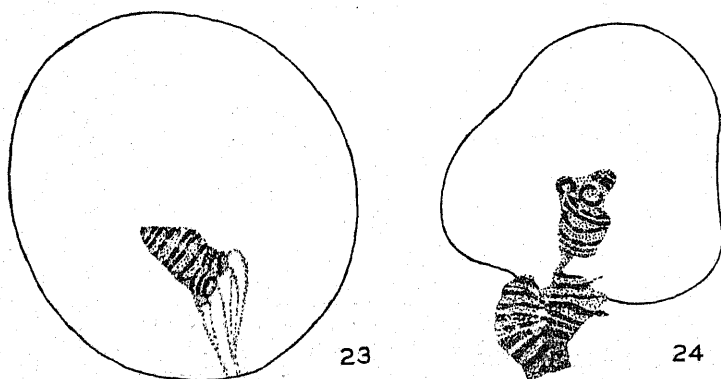


Text-Figs. 19-22. 19 Trisomic, triplo-fourth female. 20 from an XO individual. 21 an anaphase group of a tetraploid cell. 22 metaphase plate of a tetraploid cell.  $\times$  ca. 3500.

### The Salivary Chromosomes

Kikkawa (1935) first indicated that the number of strands radiating from the chromocenter in salivary gland nuclei of *D. ananassae* was six, rather than the eight to be expected from the conjugation of four pairs of V-shaped chromosomes. More recently, Kikkawa (1936) and Kaufmann (1936a) have determined independently, employing different criteria, that the six strands represent the arms of the four longer autosomes and the X-chromosomes, the fourth chromosomes being reduced to a small heterochromatin mass forming part of the chromocenter.

*The Structure of the Chromocenter*—Salivary glands were selected mostly from female larvae preparing to pupate. In the nuclei of these glands the proximal regions of the paired homologues form a compact chromocenter, from which the more distal portions of the chromosome arms radiate into the nuclear cavity (Plate 40, figs. 1, 2). The chromocenter frequently has been disrupted into its component parts (Plate 40, figs. 3, 4) by use of the method of smearing described in the first section of this paper. When the connections between the chromosome arms are thus revealed, it is found that the six strands possessing euchromatic sections represent the right and left limbs of three of the conjugated pairs of V-shaped chromosomes. The regions of these chromosomes adjacent to the primary constrictions contribute, therefore, to the formation of the chromocenter. But in addition thereto, the chromocenter discloses, following its fragmentation, a small, bipartite mass, frequently associated with the nucleolus (text-fig. 24; Plate 40, figs. 3, 4). That this heterochromatic mass represents the fourth chromosomes is suggested by the similar nucleolus-chromosome relationship of the ganglion cells. The validity



Text-Figs. 23-24. Nucleolus-satellite relationship in salivary gland nuclei of *D. ananassae*. 23 satellite with ten visible bands. 24 satellite attached to heterochromatic body representing one arm of the paired fourth chromosomes.  $\times 2250$ .

of such an interpretation receives further support from the existence in the salivary nuclei of a small, cone-shaped, banded body associated with the nucleolus, and also connected with one of the two arms of the bipartite section of the chromocenter (text-figs. 23, 24, Plate 40, figs. 3, 5, 6). The conical, banded body may be regarded, therefore, as the chromomere-like terminal segment of the longer arm of the fourth chromosomes. Its chromatic nature, together with that of the strand with which it is connected to the chromocenter, has been determined by the Feulgen technique. That the satellite represents conjugated sections of two homologous chromosomes is revealed when failure of pairing occurs in certain regions (see text-fig. 23). As indicated in text-figures 23 and 24, the satellite has about ten distinct visible bands. In some nuclei, however, the banding is less clearly defined because the chromonemata and the chromomeres are widely spaced.

The shortest of the chromosome arms with euchromatic sections represent the X-chromosomes. Such identification follows comparison of male and female cells, in the former of which the X exists in the slender haploid condition (Plate 40, fig. 3) readily distinguishable from the paired condition in the female. It is uncertain at present which of the four longer arms represent the second and third chromosomes. Experiments to determine this point are now in progress.

### Discussion

*The Chromocenter*—In *D. ananassae*, as in other species of *Drosophila*, there exists both in the resting stages of mitotic cells and in the salivary gland nuclei, that type of deeply staining chromatic region, which Heitz has designated as the chromocenter. That such regions of resting mitotic nuclei represent heteropyknotic chromosomes, or portions thereof, is evident from the contained chromonemata which they sometimes reveal (Kaufmann, 1934) as well as from their behavior through the mitotic cycle. When in close contact the heteropyknotic regions form a "Sammelchromozentrum", which is to be regarded, therefore, as a close approximation of these bodies rather than as an amorphous, vacuolated aggregate.

That the chromocenter of the salivary gland nucleus represents the heteropyknotic regions of the mitotic chromosomes was suggested by Heitz (1933a). There is at present, however, no uniformity of opinion concerning the structure of the chromocenter of the salivary gland nucleus. The concept of an amorphous, or undifferentiated chromocenter has been presented (Heitz, 1933a, 1933b; Painter, 1935; Painter and Stone, 1935; Koller, 1935). Contrasting with such inter-

pretations are those involving specific organization within the chromocenter. Several of the recent studies have indicated that the chromocenter is composed primarily of the proximal regions of the contributing chromosomes. Such structure is especially well demonstrated by dissociation of the chromocenter following pressure in smearing (Frolowa, 1936; Bauer, 1936c; Plate 40, fig. 4 of the present paper). The pressure not only separates the chromosomes from each other but reveals the heterochromatic connections between the arms of the V-shaped members of the complex. At the same time the chromomeric, banded nature of the heterochromatin is disclosed most strikingly (cf. Prokofjeva-Belgovskaja, 1935a, 1935b; Muller and Prokofjeva, 1936; Frolowa, 1936; Bauer, 1936b, 1936c). Bauer's searching analysis of this problem reveals that the heterochromatic regions of the chromosomes of *Drosophila* and the Chironomidae contain the same number of chromonemata as the euchromatic, but that heterochromomeres differ in structure from the euchromomeres.

The fourth chromosomes of ganglion cells of *D. ananassae* are unique among the autosomes of species of *Drosophila* so far described in that they are totally heteropyknotic, and stand, therefore, as an exception to the generalization that sex chromosomes are heteropyknotic to a far greater extent than the autosomes of the same group (cf. Heitz, 1935). Moreover, the fourth chromosomes are essentially "inert", since only three linkage groups have been described in *D. ananassae*, those of the X-chromosomes and of the two pairs of longer autosomes (Moriwaki, 1935; Kikkawa, 1936). In salivary gland nuclei, however, the fourth chromosomes are represented by a sizeable mass of heterochromatin, plus the satellite, contrasting thereby with the more restricted expression of such "inert" chromosomes as the Y of this and other species. If the amount of banding is an indication of the genic content of heterochromatic regions, as Frolowa (1936) has suggested, it is probable that further genetic studies will reveal genes in the fourth chromosomes. The satellite alone possesses at least ten bands, and many others exist in the arms which form part of the chromocenter. It would not to be expected that if the fourth chromosomes were totally inert, they would have been retained in all populations of a species so widely distributed as *D. ananassae*. It is now known that the term "inert" is purely relative, and is essentially a misnomer as applied to heterochromatin (cf. Schultz, 1936).

The exact portions of the fourth chromosomes which form the banded heterochromatin of the salivary gland nucleus could not be determined. The general organization of the chromocenter suggests that the spindle fiber attachment regions of the chromosomes may be

represented. In addition the satellite and the adjoining region of the long arms seem to be present. If this interpretation is correct, it is interesting to note that the satellite, which comprises about one fifteenth to one twentieth of the length of the mitotic prophase chromosome, is in the salivary gland nucleus more than half as long as the remainder of the long arm.

Kikkawa (1936) refers to the fourth chromosomes of his material as J-shaped, suggesting thereby the existence of a type differing from the V-shaped chromosomes of the present study. The occurrence of different types of Y-chromosomes in *D. ananassae* has been indicated in an earlier publication (Kaufmann, 1936a). That considerable variation may occur in the shape of the "inert" Y-chromosome of a species, has been shown by Dobzhansky's studies of *D. pseudoobscura*.

*The Position of the Nucleolus*—The existence of nucleoli in the autosomes of *D. ananassae* contrasts with the situation in other species of *Drosophila* in which their development occurs in the sex chromosomes. Association of the nucleoli with the X- and Y-chromosomes in mitotic cells of *Drosophila* was first reported for *D. melanogaster* (Kaufmann, 1933, 1934), and independently by Heitz for this and other species (1933a, 1933b). Although in *D. ananassae* the Y-chromosome possesses a nucleolus-forming region, the X-chromosome normally is not associated with the nucleolus. It is tempting, therefore, to postulate that this unique situation may have resulted from translocation of the nucleolus-forming region from the X to an autosome during the processes of speciation. But beyond the fact that the nucleoli of all species of *Drosophila* so far studied develop in heteropyknotic regions of chromosomes, there is little evidence to support such an assumption. Heitz (1933b) has emphasized that the chromosome-nucleolus relationship is independent of heteropyknosis and of the nature of the sex chromosomes, and Bauer's studies lead him to the conclusion that fundamentally many chromosome sections have the capacity for nucleolus formation (1933b).

In mitotic cells delicate chromatic threads connect those parts of the chromosome dissociated by the development of the nucleolus. That similar strands exist in the salivary gland nuclei of *Drosophila* has been reported prior to the present publication (Heitz, 1934; Kaufmann, 1934; Frolowa, 1936). When tested by the Feulgen method, I have found, as has Frolowa, that such threads give the characteristic reaction of chromatin, although Heitz (1935) defines them as "anukleal." On the basis of Bauer's recent studies, the threads may be regarded as aggregates of chromonemata. The connection between the chromosome and its satellite in the salivary gland nucleus of *D. ananassae*, for example, appears frequently as a loose

association of individual threads. In the satellite also, the nature of distribution of the chromonemata of the homologues, with their constituent chromomeres, determines whether the aspect of banding, or some less precise pattern, results.

The nucleolus-chromosome relationship of salivary gland nuclei seems to be defined less clearly in other species of *Drosophila*. Painter (1934) notes that in *D. melanogaster* none of the elements shows a constant association with the plasmosome. Frolowa (1936) finds that the nucleoli of *D. virilis*, *D. funebris*, and *D. melanogaster* are united with the small chromocenter, and through it to all the chromosomes, although in *D. funebris* the union seems at times to be between the nucleolus and the inert part of the X-chromosome. The latter situation conforms with that of *D. ananassae* and such Diptera as *Bibio* (Heitz and Bauer, 1933), *Simulium* (Geitler, 1934), and *Chironomus* (Bauer, 1935) in which the nucleolus of the salivary gland cell is associated with a designated section of a certain chromosome.

*The Constrictions*—Pronounced similarities exist between certain of the chromosomes of *D. ananassae* and those of *D. melanogaster*. Thus, of the three secondary constrictions which have been identified in each arm of the third chromosomes of *D. melanogaster* (Kaufmann, 1934), the subterminal ones alone are not pronounced in *D. ananassae*, although intimations of their existence have been encountered. More striking is the resemblance between the pronounced submedian constriction in the left arm of the second chromosome of *D. melanogaster* (Kaufmann, 1933, 1934; Prokofjeva, 1935), and a similar secondary constriction in one arm of the B chromosomes of *D. ananassae*. That such cytological evidences as similarity of form of chromosomes and positions of constrictions are inadequate, however, as a test of similarity of genic content has been emphasized in the recent studies of Dobzhansky (1935b) and Dobzhansky and Tan (1936). Despite striking similarity of the somatic metaphase chromosomes of females of *D. pseudoobscura* and *D. miranda*, the salivary glands show that not a single chromosome of *miranda* is identical with any chromosome of *pseudoobscura*. In light of such information it seems extremely hazardous, as Dobzhansky and Tan, and Bauer have emphasized, for cytologists to continue to utilize topographical features of metaphase chromosomes as the sole criteria in postulating similarities between the chromosomes of different species.

### Summary

- 1) The chromosome complement of *D. ananassae*, studied in the neurocytes, consists of three pairs of V-shaped autosomes and

the sex chromosomes. These are a pair of V-shaped X-chromosomes in the female, an X and a J-shaped Y-chromosome in the male. The chromosomes may be distinguished by relative sizes and characteristic constrictions.

2) The fourth chromosomes and the Y-chromosome appear totally heteropyknotic in resting and early prophase stages of mitotic cells. Short heteropyknotic regions lie adjacent to the spindle attachment regions of the X-chromosomes and the four longer autosomes.

3) In salivary gland nuclei there are but six chromosome arms with euchromatic sections. Four of these represent the paired arms of the longer autosomes, the other two the X-chromosome arms. The fourth chromosomes are represented in salivary gland nuclei by a small bipartite mass of heterochromatin which forms part of the chromocenter.

4) In mitotic prophases of female larvae the fourth chromosomes are associated with the nucleolus, which separates a small satellite-like portion from the remainder of the long arm of these chromosomes. In the male the Y-chromosome forms a third member of the group associated with the nucleolus.

5) The fourth chromosome-nucleolus-satellite relationship is also evident in salivary gland nuclei, the satellite appearing as a banded body associated with the nucleolus, and also connected with the fourth chromosomes by chromatic strands.

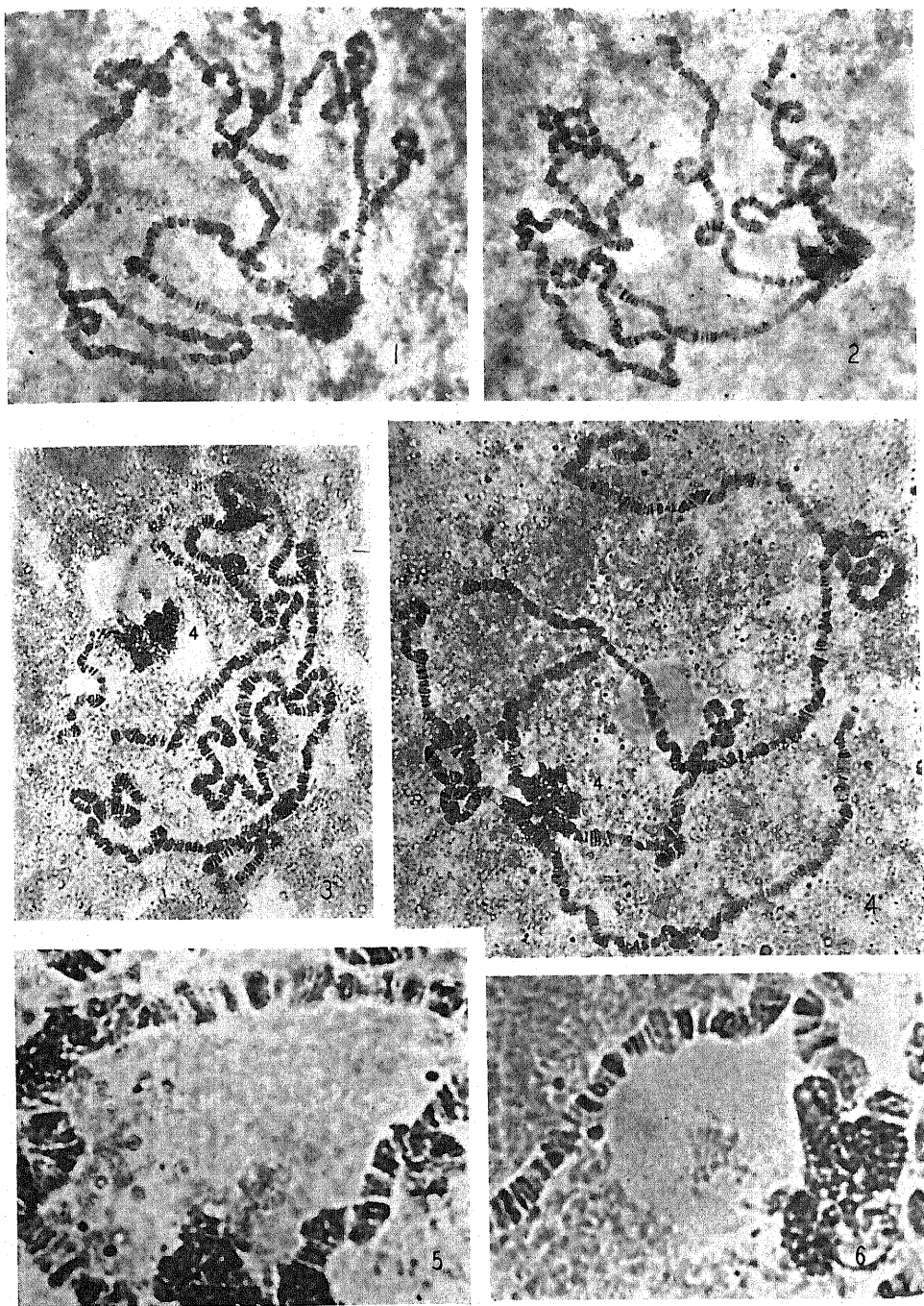
6) The chromocenter of mitotic cells and of the salivary gland cells is not amorphous, but discloses, under suitable conditions, the limits of the component chromosomes.

#### Bibliography

- Bauer, H. 1935. Der Aufbau der Chromosomen aus den Speicheldrüsen von *Chironomus Thummi* Kieffer. Zeit. Zellforsch. u. mikr. Anat. 23: 280-313.
- 1936a. The structure of the salivary gland chromosomes in Chironomidae. Amer. Naturalist 70: 37.
- 1936b. Beiträge zur vergleichenden Morphologie der Speicheldrüsenchromosomen. Zool. Jahrb. Abt. allg. Zool. u. Phys. 56: 239-276.
- 1936c. Structure and arrangement of salivary gland chromosomes in *Drosophila* species. Proc. Natl. Acad. Sci. 22: 216-222.
- Dobzhansky, Th. 1935a. The Y chromosome of *Drosophila pseudoobscura*. Genetics 20: 366-376.
- 1935b. *Drosophila miranda*, a new species. Genetics 20: 377-391.
- and C. C. Tan. 1936a. A comparative study of the chromosome structure in two related species, *Drosophila pseudoobscura* and *D. miranda*. Amer. Naturalist 70: 47-48.
- and — 1936b. Studies on hybrid sterility. III. A comparison of the gene arrangement in two species, *Drosophila pseudoobscura* and *Drosophila miranda*. Zeit. ind. Abs. u. Vererbungs. 72: 88-114.



- Frolowa, S. L. 1936. Struktur der Kerne in den Speicheldrüsen einiger *Drosophila*-Arten. Biol. Zhurnal 5: 271-292.
- Geitler, L. 1934. Die Schleifenkerne von *Simulium*. Zool. Jahrb. Abt. allg. Zool. u. Phys. 54: 237-248.
- Heitz, E. 1933a. Über totale und partielle somatische Heteropyknose, sowie strukturelle Geschlechtschromosomen bei *Drosophila funebris*. Zeit. Zellforsch. u. mikr. Anat. 19: 720-742.
- 1933b. Die somatische Heteropyknose bei *Drosophila melanogaster* und ihre genetische Bedeutung. Zeit. Zellforsch. u. mikr. Anat. 20: 237-287.
- 1934. Über  $\alpha$ - und  $\beta$ -Heterochromatin sowie Konstanz und Bau der Chromomeren bei *Drosophila*. Biol. Zbl. 54: 588-609.
- 1935. Chromosomenstruktur und Gene. Zeit. ind. Abs. u. Vererbungsl. 70: 402-447.
- and H. Bauer. 1933. Beweise für die Chromosomennatur der Kernschleifen in den Knäuelkernen von *Bibio hortulanus* L. Zeit. Zellforsch. u. mikr. Anat. 17: 67-82.
- Kaufmann, B. P. 1933. Interchange between X- and Y-chromosomes in attached X females of *Drosophila melanogaster*. Proc. Natl. Acad. Sci. 19: 830-838.
- 1934. Somatic mitoses of *Drosophila melanogaster*. Jour. Morph. 56: 125-155.
- 1936a. The chromosomes of *Drosophila ananassae*. Science 83: 39.
- 1936b. A terminal inversion in *Drosophila ananassae*. Proc. Natl. Acad. Sci. 22: 591-594.
- Kikkawa, H. 1935. An inference as to the constitution of X-chromosome in *Drosophila*. Proc. Imp. Acad. 11: 62-65.
- 1936. Chromosomes of *Drosophila ananassae* (a preliminary note). Japan. Jour. Genetics 12: 65-66.
- Koller, P. C. 1935. The internal mechanics of the chromosomes. IV. Pairing and coiling in salivary gland nuclei of *Drosophila*. Proc. Roy. Soc. B. 118: 371-396.
- Metz, C. W. 1916. Chromosome studies on the Diptera. III. Additional types of chromosome groups in the Drosophilidae. Amer. Naturalist 50: 587-599.
- Moriwaki, D. 1935. Some mutant characters in *Drosophila ananassae*. Genetica 17: 32-46.
- Muller, H. J. and A. A. Prokofjeva. 1935. The structure of the chromonema of the inert region of the X-chromosome of *Drosophila*. C. R. Acad. Sci. U. S. S. R. 1: 658-660.
- Painter, T. S. 1934. A new method for the study of chromosome aberrations and the plotting of chromosome maps in *Drosophila melanogaster*. Genetics 19: 175-188.
- 1935. The morphology of the third chromosome in the salivary gland of *Drosophila melanogaster* and a new cytological map of this element. Genetics 20: 301-326.
- and W. Stone. 1935. Chromosome fusion and speciation in *Drosophila*. Genetics 20: 327-341.
- Prokofjeva, A. A. 1934. Morphologische Struktur der Chromosomen von *Drosophila melanogaster*. C. R. Acad. Sci. U. S. S. R. 4: 102-107.
- 1935. Morphologische Struktur der Chromosomen von *Drosophila melanogaster*. Zeit. Zellforsch. u. mikr. Anat. 22: 255-262.
- Prokofjeva-Belgovskaja, A. A. 1935a. Y chromosome in salivary glands of *Drosophila*. C. R. Acad. Sci. U. S. S. R. 3: 365-366.
- 1935b. The structure of the chromocenter. Cytologia 6: 438-443.
- Schultz, J. 1936. Variegation in *Drosophila* and the inert chromosome regions. Proc. Nat. Acad. Sci. 22: 27-33.
- Tan, C. C. 1935a. Identification of the salivary gland chromosomes in *Drosophila pseudoobscura*. Proc. Nat. Acad. Sci. 21: 200-202.
- 1935b. Salivary gland chromosomes in the two races of *Drosophila pseudoobscura*. Genetics 20: 392-402.
- Yasui, K. 1935. On the structure of the chromosome in the salivary gland cell. Cytologia 6: 330-336.



Kaufmann: Morphology of the Chromosomes of *Drosophila ananassae*



## Description of Plate 40

Photomicrographs of salivary gland chromosomes of *Drosophila ananassae*. Figs. 1, 2, 4, 5 from female larvae; figs. 3 and 6 from male larvae.

Figs. 1 and 2—Showing the six chromosome arms with euchromatic sections. Four of the six arms are associated at the chromocenter; the other two have been separated. The paired X-chromosomes are represented by the two shortest arms; the four longer arms represent chromosome pairs A and B.

Fig. 3—Chromocenter dissociated by pressure in smearing. The totally heterochromatic fourth chromosomes (4) and the appended satellite are associated with the nucleolus. The unpaired X, left center and crossing the nucleolus in the photograph, exists in the slender haploid condition characteristic of the male.

Fig. 4—Separation of the components of the chromocenter following smearing. The continuity of the arms of the chromosomes is demonstrated. In smearing, the nucleolus has been dragged some distance from the fourth chromosomes (4).

Figs. 5 and 6—The nucleolus and the satellite associated with that portion of the chromocenter representing the fourth chromosomes.

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## Chromosome Variation in the Progeny of Triploid

### *Lilium tigrinum*<sup>1)</sup>

By

**Masayosi Satô**

Tokyo Imperial University

For *Lilium tigrinum* Ker-Gawl. two kinds of chromosome numbers have been recorded. Coulter, Chamberlain and Schaffner (1897), Schaffner (1906), Belling (1928) and Siang (1932) observed 12 meiotic chromosomes, while recently Takenaka and Nagamatsu (1930) first found a triploid tiger lily having 36 somatic chromosomes which number was confirmed by Satô (1932), Sass (1934), Mather (1935) and others. From this it has become evident that this plant contains both diploid and triploid forms. In Japan however no diploid tiger lilies have ever been found, as far as the writer is aware. Concerning the fertility of this lily two different views have also been proposed. Elwes (1880) early stated that "I saw never the capsule and seeds though they are figured by Nees von Eisenbeck..." Recently Wilson (1926) reported also that "During my travels in Far East I never saw *Lilium tigrinum* bearing fruit." Stout (1926) secured more than 200 bulbs of the lily from various sources and made a large number of both self- and inter-pollinations. In no case however did even a capsule start to develop, which proved that this plant was entirely self- and inter-sterile. Takenaka and Nagamatsu (1930) stated "the cause of sterility in *Lilium tigrinum* is ascribed to the irregular meiosis in the formation of non-viable pollen grains with abnormal chromosomes or gene complements". But Stout (1933) interpreted the cause of sterility from the physiological view point that the sterility of this plant is caused by self-incompatibility. On the other hand, it has been reported that it was possible to set seeds of the tiger lily. Early in 1880, Elwes stated "Mr. Hanson, of New York, informs me that he has been successful in raising seedlings from this plant, some of which differed markedly from the parent, both in forms and colour of the leaves and flowers;..." Ohga (1932) reported with an illustration the production of fruit of this plant under natural conditions. The present writer also obtained seeds of triploid tiger lily which was growing wild. The germination rate of these seeds was 8.2% (Satô 1932). Since then the writer

1) Contributions from the Divisions of Plant-Morphology and of Genetics, Botanical Institute, Faculty of Science, Tokyo Imperial University, No. 181.

conducted self- and inter-pollinations of the triploid lily every year and obtained some capsules containing both viable and non-viable seeds (Figs. 1, 2). The seeds were sown in pots which were kept

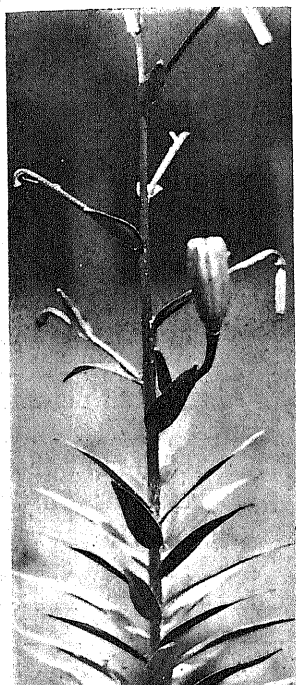


Fig. 1. A triploid *Lilium tigrinum* bearing a capsule.  $\times 1/5$ .

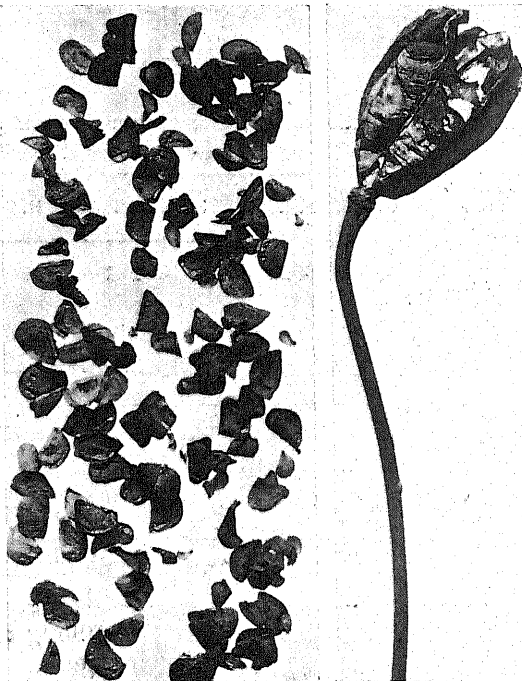


Fig. 2.  $\times 1/2$ . The same capsule as shown in Fig. 1, in its matured stage (right), together with all the seeds contained in it (left).

in the green house. Root-tips of the seedlings were fixed with Lewitsky's fluid. Paraffin sections were cut at  $18-20\ \mu$  in thickness and stained with Heidenhain's iron-alum haematoxylin or Newton's iodine gentian violet. Magnification of the text-figures  $\times 1125$ .

### Observation

*Variation in chromosome number.* As stated above the tiger lily grown in Japan has 36 somatic chromosomes and it was also found by the writer (1932) that its variety *flore-pleno* had the same number of chromosomes. Later Mather (1935) found two triploid varieties, "*Fortunae giganteum*" and "*splendens*".

By examining metaphase figures found in the root-tip cells of each of the progeny raised from the seeds obtained by the writer as mentioned above, it was proved that the chromosomes were notably variable in their number among individuals of the young tiger lilies,



ranging from 24 to 39 excepting 31 (Table 1). The number 24 is such as is found in the diploid forms, and the number 36 is nothing but that of the mother plant. Besides these, certain plants with very small chromosomes, namely fragments (f) were found; 25 + f, 26 + f, 27 + f and 28 + f (Figs. 9, 10). The frequency of the individuals with 26 or 28 chromosomes was found to be the highest (Table 1).

Table 1. Distribution of chromosome numbers in the first generation progeny of triploid *Lilium tigrinum*

Chromosome number	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	Sum	25 + f	26 + f	27 + f	28 + f	Sum
Frequency (Individuals)	4	4	16	4	14	4	8	0	5	1	1	4	11	1	1	1	79	3	1	2	2	8

From the facts obtained above it becomes evident that the first generation progeny of the triploid tiger lily consisted of at least 19 different sorts of individuals as regards their number of chromosomes, i.e., those with all the serial numbers, except 31, between 24 and 36, those with hypertriploid numbers (37, 38, 39) and those with fragments. It will be seen in the next paragraph that in the present case the same number of chromosomes does not imply constitutional similarity of the complements.

*Variation in chromosome morphology and karyotypes.* The 36 somatic chromosomes consisted of 3 long chromosomes with median constrictions, 3 long ones with sub-median constrictions and 30 medium ones with sub-terminal or terminal constrictions (cf. Satô 1932). The longest chromosomes are V-shaped with a sub-median constriction, the length difference between the arms being usually in the proportion of 3:2.

In accordance with the variation in the number of chromosomes the outward appearances of the young plants were also not uniform. The differentiation of each definite chromosome was difficult and even some of the chromosomes seemed to have changed their form and size. However the large V-chromosomes could be detected without difficulty. Taking these V-chromosomes only into consideration the progeny may be divided into two groups with respect to the chromosome complements: 1) those with the same total chromosome numbers but with a varying number of V-chromosomes; 2) those with different chromosome numbers but with the same number of V-chromosomes. The examples of the former case are shown in Figs. 4 and 7, both presenting the same complement with 26 chromosomes. The number of V-chromosomes is 4 in Fig. 4 and 5 in Fig. 7. The examples of the latter case are seen in Figs. 3-6, and Figs. 7 and 8. In Figs. 3-6, 4 complements with different chromosome numbers (25,





Figs. 3-10. Eight different chromosome complements obtained from 8 individuals of the first generation progeny of triploid *Lilium tigrinum*.  $\times 1125$ . 3,  $2n=25$ . 4 V-chromosomes (in black); 4,  $2n=26$ . 4 V's; 5,  $2n=29$ . 4 V's; 6,  $2n=34$ . 4 V's; 7,  $2n=26$ . 5 V's; 8,  $2n=28$ . 5 V's; 9,  $2n=25+f$ . 2 V's; 10,  $2n=26+f$ . 2 V's.

26, 29, 34) are shown, in each of which 4 V-chromosomes can be recognized, while Figs. 7 and 8 each show 5 V-chromosomes, though they present different complementary chromosomes (26 and 28). In some of the plants only two V's in addition to other kinds of chromosomes 23-26 in number could be observed as shown in Figs. 9 and 10, where one fragment is found respectively.

From the facts stated above it may be said that the first generation progeny of the tiger lily have attained a remarkable variation of karyotypes, though they have been considered only from the standpoint of the large V-chromosomes.

### Considerations

In the first generation progeny of the triploid tiger lily, 15 kinds of somatic chromosome numbers, ranging from 24 to 39 excepting 31, were found. 24 and 36 are the chromosome numbers of diploid and triploid forms respectively, while the numbers 37, 38, 39

are of hypertriploid type. These varying numbers must have arisen by the gametes with irregular numbers of chromosomes being produced by the autotriploid mother plant. And the production of the anormal gametes may be due to irregular meiosis or due to structural change taking place in chromosomes during the course of development of the pollen grains or embryos. Anormal formation of pollen grains in the triploid plant was already described by Takenaka and Nagamatsu (1930) and the writer (cf. Satô 1932, Fig. 3). Considering the chromosome numbers found in the progeny examined at least 16 kinds of gametes might have been formed by the mother plant. The chromosome numbers being distributed into these gametes are presumed to be from 12 to 27, and 69 combinations (karyotypes) of these distributed numbers are expected to occur. From the range of the somatic numbers mentioned above the inference may be drawn that the gametes with less chromosomes than 12 were not viable and the gametes with from 12 to 14 chromosomes were most frequently formed.

The karyotypes of the plants examined showed a remarkable variation as was expected, though in the present case the karyotypes were analyzed only from the stand point of the large V-chromosomes. It seemed that some of the chromosomes underwent a structural change since the fragments were often found in certain plants. Further examination will make clear this point among others.

According to the previous investigators (Takenaka and Nagamatsu, Mather) the triploid lilies have a number of bivalents and univalents beside trivalents; so from this fact together with the results obtained in the present work it may be considered that the triploid tiger lilies with 36 chromosomes had a certain number of chromosomes with changed structural portions besides having homologous and non-homologous chromosomes. The degree of sterility can not be said to be uniform in all living triploid lilies, though they may be clones of not so many sources, because the different constitutions of the karyotypes may act in different ways even as regards this phenomenon. The occurrence of only triploid lilies with 36 chromosomes in nature, notwithstanding the formation of the progeny with different chromosome complements as shown in this work, is at present unknown, but the viability of the plants with chromosomes other than triploid numbers or non-viable combinations of chromosomes must be taken into consideration in this connection.

### Summary

The first generation progeny raised from the seeds obtained by self- and inter-pollinations of the triploid *Lilium tigrinum* have been

examined with a view of obtaining evidence of variation of their chromosome complements. The plants examined varied markedly in their somatic numbers representing all the numbers between 24 and 39, except 31. Beside these, there were found a number of plants with fragments, i.e.  $25 + f$ ,  $26 + f$ ,  $27 + f$  and  $28 + f$ . The karyotypes were analyzed from the stand point of large V-chromosomes, which showed that the karyotypes were not uniform in these plants.

The writer is deeply indebted to Dr. Y. Sinotô who has given him kind advice and criticism. Thanks are also due to Dr. Y. Takenaka who kindly gave him some material for the present study.

Division of Genetics, Botanical Institute,  
Faculty of Science, Tokyo Imp. University

#### Reference

- Belling, J. 1928. Nodes and chiasmata in the bivalents of *Lilium* with regards to segmental interchange. Biol. Bull., 54: 465-470.
- Coulter, J. M., Chamberlain, C. J. and Schaffner, J. H. 1897. Contribution to the life history of *Lilium philadelphicum*. Bot. Gaz., 23: 412-452.
- Elwes, H. J. 1888. A monograph of the genus *Lilium*. London.
- Mather, K. 1935. Meiosis in *Lilium*. Cytologia, 6: 354-380.
- Ohga, I. 1924. Pollens and Seeds of *Lilium tigrinum*. Amaeba, 2: 320.
- Preston, I. 1926. *Lilium* from seed in Ontario. Gard. Chron., 76: 320.
- 1926. Growing lilies from seed. Horticulture, 4: 95.
- Sass, J. 1934. Chromosome fragmentation in *Lilium tigrinum* Ker. Amer. Nat., 68: 471-479.
- Satô, M. 1932. Chromosome studies in *Lilium*. Bot. Mag. (Tokyo), 46: 68-88.
- 1935. Sterility in *Lilium tigrinum*. Jap. Jour. Genet., 11: 193-194.
- Schaffner, J. H. 1906. Chromosome reduction in the microsporocytes of *Lilium tigrinum*. Bot. Gaz., 41: 183-191.
- Siang, H. 1932. Structure of somatic chromosomes in *Lilium tigrinum*. La Cellule, 41: 163-173.
- Stout, A. B. 1932. Sterility in Lilies. Journ. Hered., 13: 369-373.
- 1933. Sterility in lilies (*Lilium*). R. H. S. Lily Year Book.
- Takenaka, Y. and Nagamatsu, T. 1930. On the chromosomes of *Lilium tigrinum* Ker-Gawl. Bot. Mag. (Tokyo), 44: 386-391.
- Wilson, E. H. 1925. The lilies of Eastern Asia.

## Über eine diplo-tetraploide Chimäre bei *Triticum*<sup>1)</sup>

Von

**Kosuke Yamashita**

Kaiserliche Universität Kyoto

(Mit 2 Tafeln)

### 1. Einleitung

Während meiner Untersuchungen über 29-chromosomige trisomische Pflanzen in der Nachkommenschaft pentaploider Weizenbastarde habe ich ein Individuum gefunden, welches in ganzen Antheren bzw. Antherenfächern P.M.Z. mit verdoppelter Chromosomenzahl ( $2n = 58$ ) aufwies. Näheres über diese Chromosomenchimäre soll im folgenden mitgeteilt werden.

### 2. Material und Methoden

Die trisomische 29-chromosomige Pflanze, die sich als Chimäre erwies, trat im Jahre 1936 in der Nachkommenschaft Nr. 17 auf, die sich von einem 29-chromosomigen Individuum aus der Rückkreuzungsbastardsverbindung (*Triticum polonicum* L. var. *vestitum* KÖRN.  $\times$  *T. spelta* L. var. *Duhamelianum* KÖRN.)  $F_1 \times T. polonicum$  L. var. *vestitum* KÖRN. ableitete (vgl. KIHARA und WAKAKUWA, 1935 u. YAMASHITA, 1937 a, b). Ihre Feldnummer war 17/41.

Bei der Untersuchung der Meiosis wurden Dauer- und Schmierpräparate der P.M.Z. benutzt. Das Material für die ersteren wurde mit CARNOY-FLEMMING nach KIHARAS Vorschrift fixiert und mit NEWTON's Gentianaviolett gefärbt. Die Dicke der Paraffinschnitte betrug  $18 \mu$ . Die Schmierpräparate wurden meistens nach BELLINGS Verfahren angefertigt. Manchmal habe ich mit gutem Erfolg die Antheren in CARNOY's Flüssigkeit zerdrückt und den auseinandergeschmierten Inhalt mit Eisenessigkarmin behandelt (vgl. YAMASHITA, 1937c).

Für die Abbildungen und Mikrophotos wurden meist Schmierpräparate verwendet.

### 3. Meiosis

Die diplo- und tetraploiden P.M.Z. traten bei 17/41 stets getrennt

1) Contributions from the Laboratory of Genetics, Biological Institute, Kyoto Imperial University, No. 83.

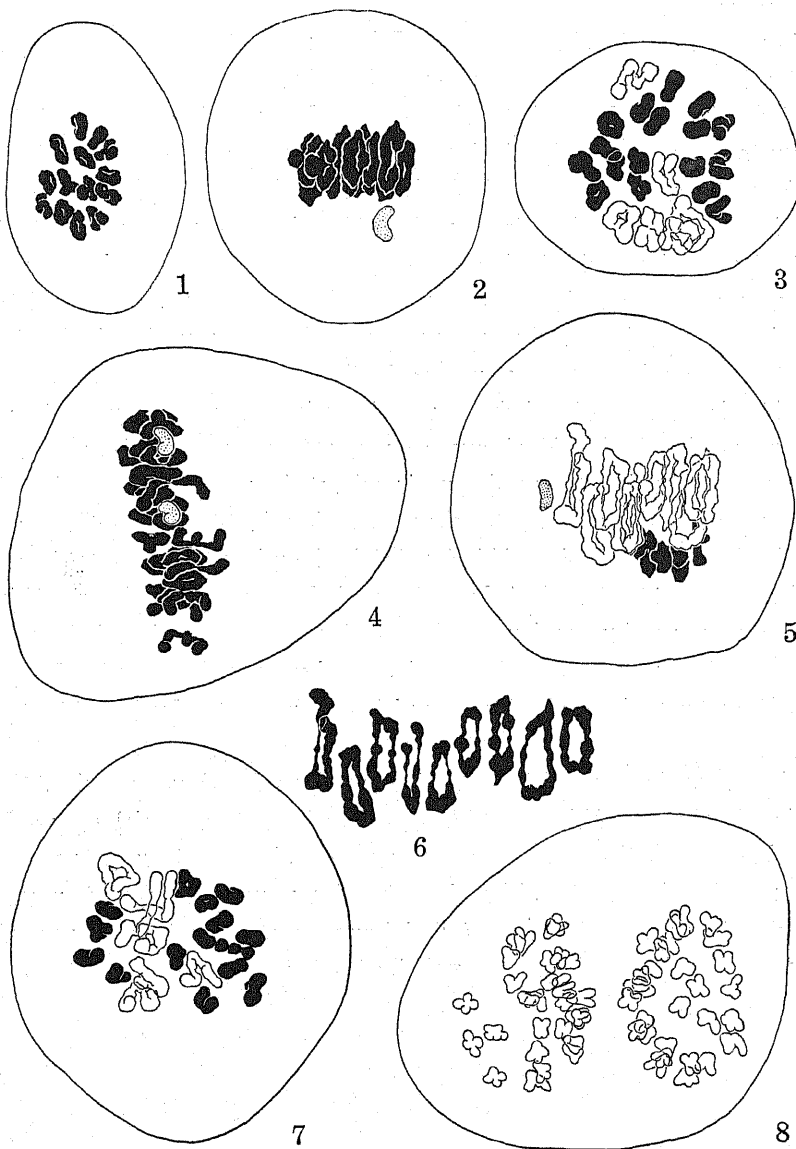


Abb. 1-8. 1. 28-chromosomige Pflanze. I. Metaphase in Polansicht;  $14_{II}$ . Vergr. ca. 1080-fach. 2. Trisomische 29-chromosomige Pflanze. I. Metaphase in Seitenansicht;  $14_{II}+1_I$ . Vergr. ca. 1080-fach. 3-7. 17/41. I. Metaphase in P.M.Z. mit doppelter Chromosomenzahl (58). Vergr. ca. 1080-fach. 3. Polansicht;  $6_{IV}+17_{II}$  (Tetralvalente weiß, Gemini schwarz). Vgl. Mikrophoto Taf. 41, 7. 4. Seitenansicht; wahrscheinlich  $28_{II}+2_I$ . Vgl. Mikrophoto Taf. 41, 6. 5. Seitenansicht;  $9_{IV}+$  einige Gemini  $+1_I$ . Vgl. Mikrophoto Taf. 42, 12. 6. Die Quadrivalenten der Abb. 5. 7. Polansicht;  $7_{IV}+15_{II}$ . Vgl. Mikrophoto Taf. 42, 11. 8. 17/41. I. Anaphase in einer P.M.Z. mit doppelter Chromosomenzahl. Die beiden Tochtergruppen enthalten je 29 Chromosomen. Vgl. Mikrophoto Taf. 41, 6 und 42, 13 a, b. Vergr. ca. 1080-fach.

auf, in verschiedenen Ährchen, Blütchen, Antheren oder Antherenfächern (Mikrophoto Taf. 41, 4). P.M.Z. mit beiderlei Chromosomenzahlen habe ich nie in ein und demselben Antherenfach zusammen gefunden. Die Antheren bzw. Antherenfächer mit tetraploiden P.M.Z. waren auf den ersten Blick durch die Größe dieser Zellen, die bedeutend größer waren als die diploiden, erkennbar.

Die Meiosis verlief in den diploiden P.M.Z. wie erwartet, d.h. es traten in der I. Metaphase  $14_{II} + 1_I$  auf, ebenso wie bei den 29-chromosomigen Geschwisterpflanzen (Abb. 2), deren Meiosis sich von derjenigen der 28-chromosomigen (Abb. 1) nur durch das Vorkommen eines Univalenten unterschied.

Die Meiosis in den P.M.Z. mit doppelter Chromosomenzahl war vor allem durch das Vorkommen von tetravalenten Chromosomenverbänden charakterisiert, deren Anzahl recht variabel war. In Abb. 3 ist eine P.M.Z. mit der Chromosomenkombination  $6_{IV} + 17_{II} = 58$  zu sehen (vgl. Taf. 41, 7); Abb. 7 zeigt die Kombination  $7_{IV} + 15_{II} = 58$  (vgl. Taf. 42, 11). Abb. 5 bringt eine P.M.Z. mit  $9_{IV}$ , die in Abb. 6 noch einmal wiedergegeben sind (vgl. Taf. 42, 12). Die P.M.Z. der Abb. 4 stellt einen der seltenen Fälle ohne Quadrivalente dar; sie enthält außer 2 Univalenten (punktiert) nur Bivalente ( $28_{II} + 2_I$ ) (vgl. Taf. 41, 6).

Die Anaphase macht in der Regel einen durchaus regelmäßigen Eindruck, wie aus Abb. 8 (vgl. Taf. 41, 6; Taf. 42, 13 a, b) zu ersehen ist. Jede der beiden Tochtergruppen enthält 29 Chromosomen, die sich mit Leichtigkeit zählen lassen. Hier und da findet man in diesem Stadium in der Äquatorialebene verzögerte Univalente oder Fragmente (Abb. 9 bzw. 10; vgl. Taf. 41, 7).

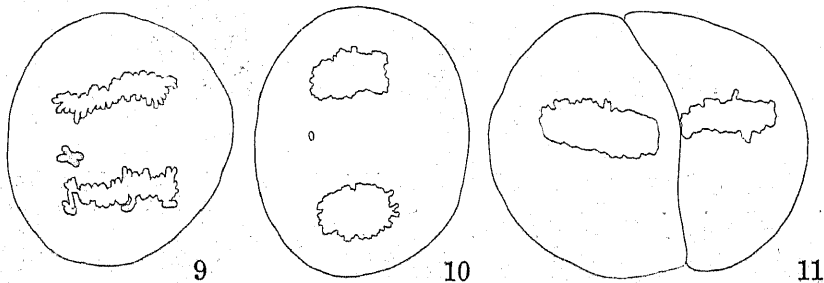


Abb. 9-11. 9-10. 17/41. Telophase in P.M.Z. mit doppelter Chromosomenzahl. Vergr. ca. 800-fach. 9. Ein verzögertes Univalentes. Vgl. Mikrophoto Taf. 41, 7. 10. Ein Fragment in der Äquatorialebene. Vgl. Mikrophoto Taf. 41, 7. 11. 17/41. II. Metaphase in Seitenansicht, Sehr regelmäßige Kernplatte. Vergr. ca. 800-fach.

#### 4. Kerneinwanderung bei 17/41

Regelmässige echte Kerneinwanderung (vgl. KIHARA und LILIENFELD, 1934) erschien bei dieser Pflanze; sie sei hier erwähnt.

Abb. 13 bringt eine große tetraploide P.M.Z. (*d*) in I. Metaphase; sie enthält außer  $2_I$  (punktiert) dünne, zum Teil degenerierende Chromosomen, die hier in einem früheren Stadium eingewandert sind. In der nächsten Umgebung der großen Zelle liegen 3 kleine Restzellen *a*, *b* und *c* (vgl. Mikrophoto Taf. 42, 8), deren jede  $4_{II} + 8_I$  enthält.

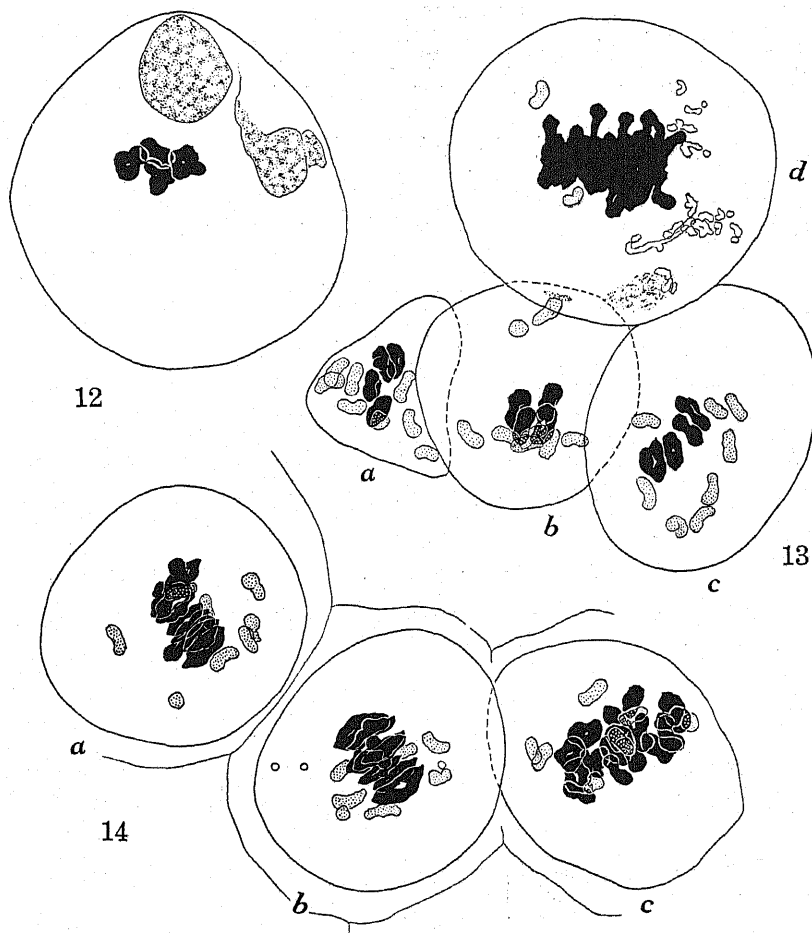


Abb. 12-14. 17/41. P.M.Z. mit Kerneinwanderung. Vergr. ca. 1080-fach. 12. Eine Restzelle mit  $4_{II}$ ; außerdem 2 Kerne in Ruhe. Vgl. Mikrophoto Taf. 42, 10. 13. *a*, *b* und *c*. Drei Restzellen mit je  $4_{II} + 8_I$ . Daneben P.M.Z., *d*, die außer einer verdoppelten Chromosomengarnitur eingewanderte Elemente enthält. Vgl. Mikrophoto Taf. 42, 8. 14. Drei nebeneinanderliegende P.M.Z. *a*,  $8_{II} + 8_I$ . *b*,  $8_{II} + 7_I + 3Fr.$  *c*.  $(14_{II} + 1_I) + (2_{II} + 6_I)$ , die  $2_{II}$  und  $6_I$  sind eingewandert. Vgl. Mikrophoto Taf. 42, 9.



Ähnliches bietet Abb. 14 (vgl. Taf. 42, 9); die Chromosomen-garnituren der Zellen *a*, *b* und *c* sind:

$$a \dots 8_{II} + 8_I$$

$$b \dots 8_{II} + 7_I + 3 \text{ Fr.}^1)$$

$$c \dots 16_{II} + 7_I = (14_{II} + 1_I) + (2_{II} + 6_I)$$

Es ist bemerkenswert, daß die kleinen Restzellen in jedem Fall dieselbe Chromosomenzusammensetzung aufwiesen und daß sie keine Degenerationserscheinungen aufwiesen. Die Gesamtzahl der Chromosomen der Restzellen war 48; sie dürfte es sein, die die eingewanderten Elemente (10 Chromosomen) geliefert haben.

In Abb. 12 sind nur 4 sich normal darstellende Bivalente zu sehen und, außerdem, 2 Kerne im Ruhezustand (vgl. Taf. 42, 10).

### 5. Morphologie und Fertilität von 17/41

Wie Abb. 15 zeigt, bestand keine merkbarer Größenunterschied zwischen 17/41 und den diploiden Schwesterpflanze.

Auch die Stomata auf der Blattunterseite waren gleich groß (Taf. 41, 1, 2 und 3).

Ebensowenig konnte ein Unterschied in der Ährenlänge oder Ährendichtheit festgestellt werden. Betreffs der Ährengestalt traten in der Nachkommenschaft Nr. 17 drei Typen auf, die in Abb. 16 als I, II und III bezeichnet sind.<sup>2)</sup> Die Ähren von 17/41 gehörten zum heterozygotischen Typus II und waren von den Ähren anderer 29-chromosomiger Geschwister des gleichen Typus nicht zu unterscheiden.

Ein deutlicher Unterschied tritt nur in der Größe der P.M.Z. und der Pollenkörner zutage, wie aus Mikrophoto Taf. 41, 4 ohne weiteres zu ersehen ist.

Bei der Bestäubung der 28-chromosomigen Pflanze mit Pollen von 17/41 habe ich 100-prozentigen Ansatz



Abb. 15. Links 17/41, rechts eine 28-chromosomige Schwesterpflanze. Aufgenommen am 10. Juni, 1936. 1/12 Nat. Gr.

1) Fr. = Fragment.

2) Es wurde in Nr. 17 eine einfache Spaltung betreffs der Ährengestalt festgestellt.

erzielt. Allerdings kann ich nicht sagen, welchen Anteil die doppelchromosomigen Riesenpollen in der von mir zu diesem Versuch benutzten Pollenprobe hatten.

Auch auf der weiblichen Seite war die Fertilität sehr gut. Tab. 1 bringt die Fertilitätszahlen für je 2 28- und 29-chromosomigen Pflanzen der Nr. 17 und für 17/41. Ob und inwiefern auch in den Embryosäcken Chromosomenverdoppelung stattgefunden hat kann ich vorläufig noch nicht sagen.

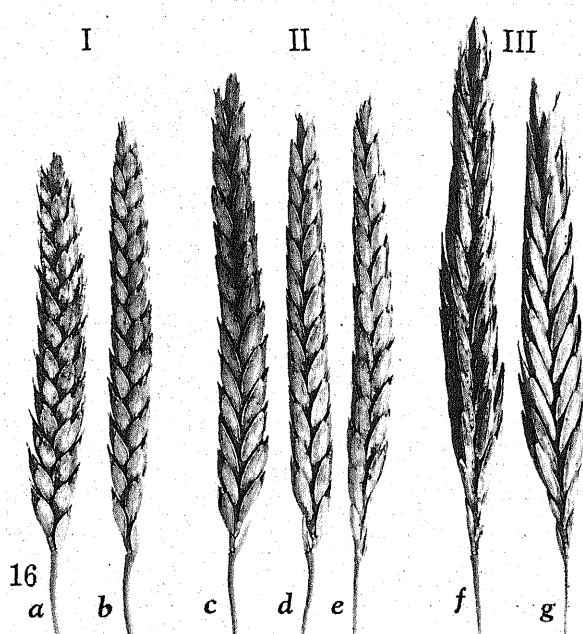


Abb. 16. Ährentypen aus Nr. 17. I und III homo-, II heterozygotisch. a, c und f, Ähren 28-chromosomiger Pflanzen. b, d und g, Ähren 29-chromosomiger Pflanzen. e, Ähre von 17/41.

Tab. 1. Körneransatz

Pflanzen-Nr.	Chromosomenzahl (2n)	Körneransatz in %
17/ 6	28	79.54
/40	28	95.83
/24	29	95.65
/17	29	75.00
/41	Chimäre von 29 und 58	86.00
		91.66

## 6. Ergebnisse und Schlußbetrachtung

Die Meiosis in den tetraploiden P.M.Z. von 17/41 war vor allem durch das Auftreten von Quadivalenten charakterisiert. Durch diesen

Befund ist sichergestellt, daß die betreffenden P.M.Z. eine verdoppelte Chromosomengarnitur besaßen.

Die Chromosomenzahlen konnten nur in den P.M.Z. bestimmt werden. Angesichts dessen, daß die Pflanze trotz ihrer Chimärenatur sich durchaus einheitlich darstellte und die gleichen Größenverhältnisse aufwies wie die 29-chromosomigen Schwesterpflanzen, ist es sehr wahrscheinlich, daß die Chromosomenverdoppelung erst im Archespor, vor der Differenzierung der P.M.Z. stattgefunden hat.

Es liegen in der Literatur zahlreiche Angaben über Verdoppelung

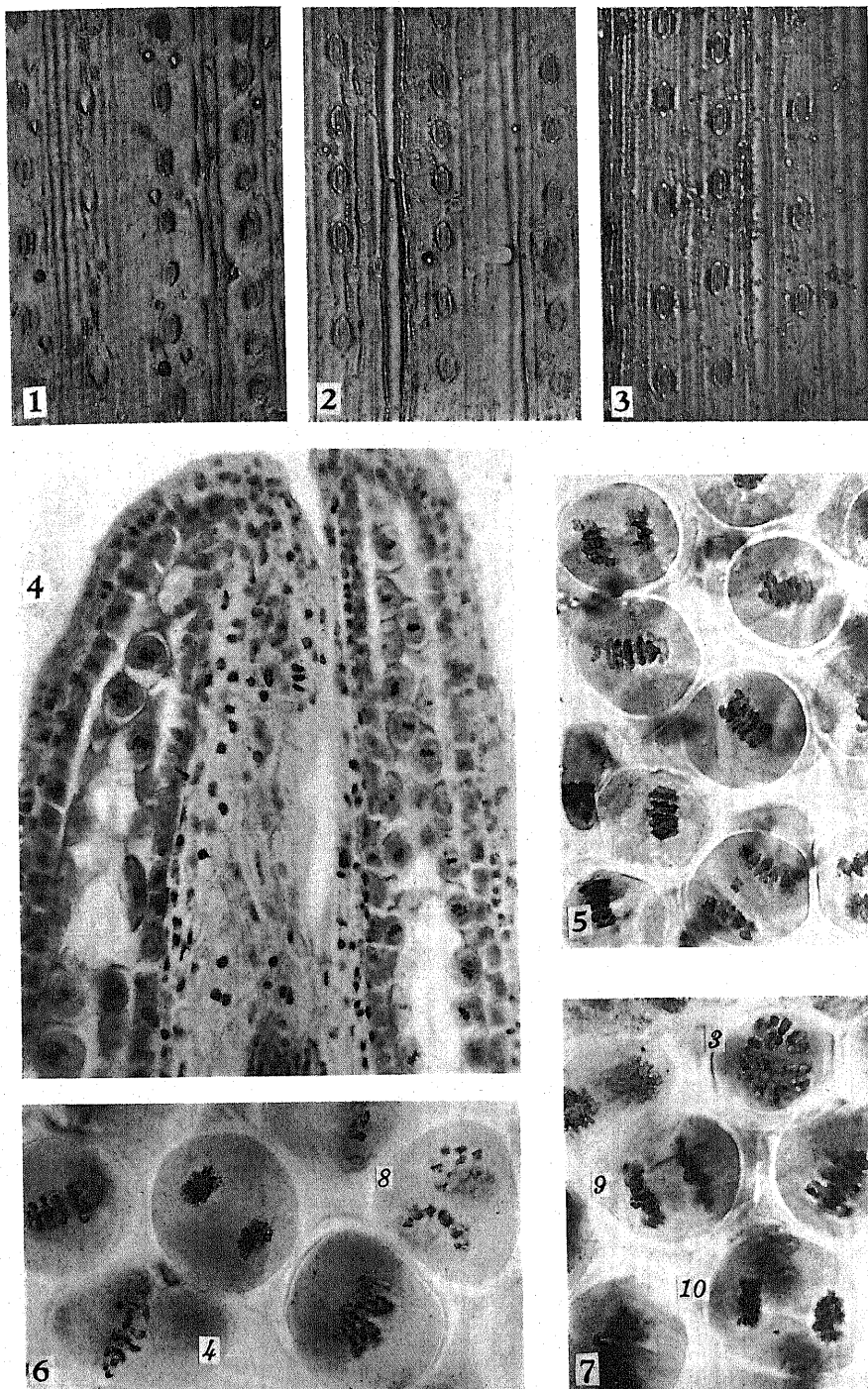
der Chromosomen vor. Die Meinung betreffs des Stadiums, auf welchem dieser Prozeß vor sich geht, sind geteilt. Z.B. WINGE (1917, 1932) legt das Hauptgewicht auf Verdoppelung im somatischen Gewebe, ohne einen Beweis dafür zu erbringen, während ROSENBERG (1926/27) auf Grund seiner Befunde vor allem in der Regression der Gonotokonten den Weg zur Erhaltung polyploider Chromosomenzahlen erblickt. KIHARA und KATAYAMA (1931) sind auf Grund ihrer Untersuchungen an *Aegilotricum* zu der Überzeugung gekommen, daß die von ihnen hergestellte Pflanze durch Regression entstanden ist. Sie ziehen aber auch die Möglichkeit der Entstehung durch Chromosomenverdoppelung im somatischen Gewebe des Embryos in Betracht. Es kann keinem Zweifel unterliegen daß vielfache Chromosomenzahlen auf beiden Wegen entstehen können (vgl. NEWTON und PELLEW, 1929). Es ist nur viel schwerer, oft sogar unmöglich, den Prozeß im somatischen Gewebe zu erfassen.

Die von mir untersuchte Pflanze 17/41 stellt den ersten Fall dar, in dem der Inhalt ganzer Antheren bzw. Antherenfächer wahrscheinlich auf einem frühen Stadium der Archesporentwicklung tetraploid geworden ist.

Die vorliegende Untersuchung wurde unter Leitung von Herrn Prof. Dr. H. KIHARA ausgeführt, dem ich an dieser Stelle meinen herzlichsten Dank ausspreche.

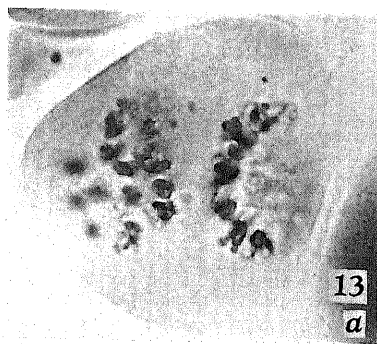
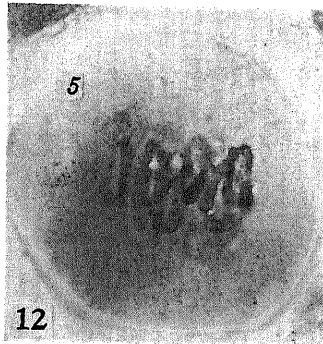
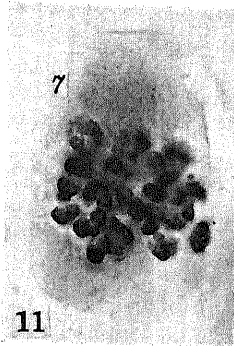
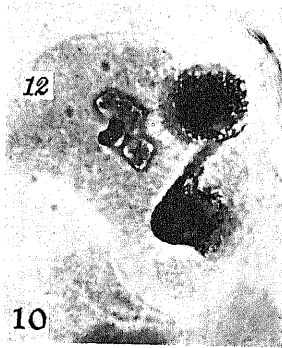
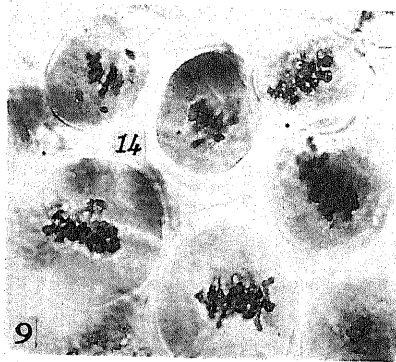
#### Zitierte Literatur

- Kihara, H. u. Katayama, Y. 1931: Genomanalyse bei *Triticum* und *Aegilops* III. Zur Entstehungsweise eines neuen konstanten oktoploiden *Aegilotricum*. Cytologia, 2.
- Kihara, H. u. Lilienfeld, F. 1934: Kerneinwanderung und Bildung syndiploider Pollenmutterzellen bei dem  $F_1$ -Bastard *Triticum aegilopoides*  $\times$  *Aegilops squarrosa*. Jap. Journ. Gen. 10.
- Kihara, H. u. Wakakuwa, Sh. 1935: Weitere Untersuchungen über die pentaploiden *Triticum*-Bastarde IV. Jap. Journ. Bot., 7.
- Newton, W. C. F. a. Pellew, C. 1929: *Primula kewensis* and its derivatives. Journ. Gen., 20.
- Rosenberg, O. 1926/27: Die semiheterotypische Teilung und ihre Bedeutung für die Entstehung verdoppelter Chromosomenzahlen. Hereditas, 8.
- Winge, Ø. 1917: The Chromosomes; their numbers and general importance. C. R. Trav. Carlsb., 13.
- 1932: On the origin of constant species-hybrids. Sv. Bot. Tid., 26.
- Yamashita, K. 1937a: Karyogenetische Studien über die pentaploiden Bastarde: Einige trisomische Nachkommenschaften mit 29 Chromosomen. (Vorläufige Mitteilung). Jap. Journ. Gen. 13.
- 1937b. Genetische Untersuchungen über den Markgehalt der Weizen-Halme. Mem. Coll. Agr. Kyoto Imp. Univ. 39.
- 1937c. Ein gewandtes Verfahren der Eisenessigkarmin-Präparate. Botany and Zoology 5.



Yamashita: Über eine diplo-tetraploide Chimäre bei *Triticum*





Yamashita: Über eine diplo-tetraploide Chimäre bei *Triticum*





## Tafelerklärung

Die kleinen kursiven Ziffern auf Fig. 6-13. entsprechen den Nummern der Textabbildungen.

## Tafel 41

- 1-3. Stomata der Blattunterseiten in Versuchsnr. 17. Vergr. ca. 90-fach. (Mikrophographien von Dauerpräparaten nach SUZUKIS Universal Mikroprinting Method, S. U. M. P.).  
1. 28-chromosomige Pflanze. 2. 29-chromosomige Pflanze. 3. 17/41.  
4. 17/41. I. Metaphase in einer Anthere mit einem normalen und einem verdoppelten Antherenfach. Links normal ( $14n+1r$ ), rechts verdoppelt (Dauerpräparat). Vergr. ca. 210-fach.  
5. I. Metaphase bei einem 29-chromosomigen Individuum. Vergr. ca. 470-fach.  
6-7. 17/41. Aus Antherenfächern mit doppelter Chromosomenzahl. Vergr. ca. 470-fach. (Vgl. Textabbildungen 3, 4, 8, 9 und 10).

## Tafel 42

- 8-10. 17/41. Kerneinwanderung. Näheres im Text. Vergr. von 8 und 9 ca. 470-fach. von 10 ca. 1020-fach. (Vgl. Textabbildungen 12, 13 und 14).  
11. 17/41. I. Metaphase in Polansicht in einer P. M. Z. mit doppelter Chromosomenzahl. Vergr. ca. 1020-fach. (Vgl. Textabbildung 7).  
12. 17/41. I. Metaphase in Seitenansicht in einer P. M. Z. mit doppelter Chromosomenzahl. Vergr. ca. 1020-fach. (Vgl. Textabbildung 5).  
13 a und b. Anaphase in zwei verschiedenen Abbildungsebenen photographiert. In beiden zusammen können 58 Chromosomen gezählt werden. Vergr. ca. 1020-fach. (Vgl. Textabbildung 8 und Tafel 41, 6).
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## Geographical Distribution of Chromosomal Prime Types in *Datura stramonium*\*

By

Albert F. Blakeslee, A. Dorothy Bergner and Amos G. Avery

Department of Genetics, Carnegie Institution of Washington,  
Cold Spring Harbor, N. Y.

The geographical distribution of plants and animals has received considerable attention in the study of evolution of species. Since we believe the problem of evolution of species can best be stated in terms of the evolution of their chromosomes, we have for some years been making a study of the chromosomal changes which have taken place in nature within the genus *Datura*. The present paper has to do with Prime Types (PT's), or races with modified chromosomes in terms of a standard race, within the single species *D. stramonium*.

At the present time we have in our collection over 90 prime types of various kinds which have been obtained from radiation and heat treatments, from aged seeds and pollen grains and from races in nature. Prime types have been discussed in an earlier publication (1). Only those obtained from nature need concern us here. That chromosomal races existed in *Datura* was early recognized from breeding behavior (2). By this means we were able to classify 198 of our strains into 73 "A" races which gave normal inheritance of the purple-white factors and 125 "B" races which gave abnormal inheritance of these factors. Belling (3) showed that the peculiarities of the so-called "B" race (now called prime type 2) could be explained by his hypothesis of segmental interchange. Since that time we have been accumulating evidence regarding the occurrence in nature of prime types in *D. stramonium* and other species of this genus.

In 1929, a preliminary report (4) was made regarding the "cryptic" chromosomal types of *D. stramonium* which up to that time had been found in nature. These prime types, as we now term them, were called cryptic because there was nothing in their appearance which could be used to distinguish their chromosomal constitution from that of our standard Line 1. They may show recognizable differences, however, due to genes. In the paper cited we were unable to distinguish all the PT's we now know and had determined the end arrangements of the modified chromosomes in only PT 2. At that

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\* "Awarded the A. Cressy Morrison prize for 1936 by the New York Academy of Sciences."

time also there was no knowledge available regarding the end arrangements of modified chromosomes in species of the genus *Datura* other than *D. stramonium*. Consequently, no relationship could be suggested between the PT's in different species. The numbers of races in our earlier paper were not sufficient to justify the plotting of their geographical distribution. Further tests enable us now to report on the chromosomal constitution of 583 races and to say something regarding their distribution in the six continents, as well as to suggest relationships between prime types and evolution in the genus *Datura*.

The method employed in testing races for prime types in *D. stramonium* was essentially the same as that used for races in other *Datura* species. An assemblage of races was secured from as widely different parts of the world as possible through our own collections and through the kindness of correspondents who cooperated in obtaining seeds for our tests.<sup>1)</sup> Each collection of seeds from a given locality which generally came from a single plant was assigned an S.I. (seed introduction) number. In our own collections it was possible to know with some precision how many plants and how many loci were represented from a given region. Our custom was to sample a patch of plants by gathering seeds from two or three individuals in the group, each receiving a separate S.I. number and becoming the starting point of a new race. Each patch may be considered a

1) The investigation is indebted for seeds of *D. stramonium* to members of the *Datura* group and to the following:—A. H. G. Alston, G. E. Anastasia, W. A. Archer, A. Aslander, B. T. Avery, C. A. Backer, A. M. Banta, P. Baranov, H. D. Barker, H. H. Bartlett, E. Baur, Mathilde Bensaude, D. Bois, R. Bolton, D. N. Borodin, S. Boshnakian, T. Brinkley, N. L. Britton, L. A. Brown, S. C. Bruner, S. Calderon, Rosalie Caples, W. M. Carne, J. de Carvalho e Vasconcellos, E. Cheel, A. Chevalier, R. Chodat, H. H. Chung, J. L. Collins, C. Conzatti, H. F. Crampton, J. N. Couch, C. B. Davenport, Gertrude C. Davenport, G. A. de Mol, P. H. Dorsett, R. A. Emerson, Y. Emoto, N. Esposta, J. M. Fogg Jr., P. Font-Quer, L. Fourton, K. Fujii, Lulu Gaiser, R. R. Gates, Edith Grosvenor, V. Goessens, G. K. Gunjko, J. W. Harshberger, L. J. Heatly, A. H. Henckel, C. M. Holmes, F. J. Hopkins, S. Horowitz, P. G. Howes, J. B. Hurry, M. A. Jabbar, L. I. Kazakevich, D. A. Keck, Anna M. Kornhauser, H. H. Laughlin, I. F. Lewis, D. H. Linder, F. E. Lloyd, A. Lutz, R. Maire, E. Manoillov, O. Mattiolo, J. R. A. McMillan, M. A. Meunissier, N. W. Monteverde, Daisie Morrison, J. Nonidez, J. F. Normand, A. Noronka, I. V. Novopokrovsky, R. T. Palhinha, G. Pallacci, R. N. Parker, Emily Perkins, Orra P. Phelps, E. P. Phillips, A. A. Pristupa, W. Razdorskij, B. E. Read, G. M. Reed, P. H. Rolfs, K. Ryerson, J. G. Salas, R. Salgues, A. Sangiovanni, Virginia Schoonmacher, A. Serck, S. Shantz, P. Shevchenko, D. K. Larinov, R. Stewart, N. Stshibrya, A. H. Sturtevant, Z. Szabo, F. Thone, N. P. Vercholanova, C. T. White, O. E. White, Anna R. Whiting, C. M. Woodworth, Elaine Young, J. L. de Zuazo, A. de Zulueta.

Botanic Garden of Tiflis, Gebruder Dippe, Institute of Applied Botany, Leningrad, U. S. Department of Agriculture.

Without the cooperation of these just mentioned it would not have been possible to obtain records from such widely distributed regions of the world. We should appreciate receiving samples of a few seeds from individual plants of *D. stramonium* from regions not represented in our tables as well as seeds of other species of *Datura* which we hope to study in a similar manner.

separate locus. Sometimes the races from a single patch differed chromosomally. In such cases the patch might be listed as the locus for several different prime types or prime type associations. When collections were sent to us it was not always certain whether the seeds in a packet came from one or from many plants, but it has been our practice to record each packet as a separate S.I. As a preliminary test of the homozygosity of the race, about a dozen plants of each S. I. are grown. Examinations are made for pollen abortion but the chromosomes are not examined in this generation. It has occasionally happened that segregation of pollen abortion types has been discovered in this first test generation. Probably segregation of PT's which are unaccompanied by pollen abortion might similarly have been discovered if it had been feasible to make the necessary cytological examinations. A more profitable procedure was found to be an examination of the chromosomes in hybrids between a selected plant of the given S. I. race and our standard Line 1 (PT 1).<sup>1)</sup> If the given plant happened to be heterozygous for a PT it would be an even chance which component appeared in the F<sub>1</sub> hybrid examined, hence the accidental choice of a heterozygous plant as the representative of the race to be tested would in no way affect the random character of the sample. If in the hybrid only twelve bivalents are observed in the first meiotic division in pollen mother cells, the S. I. in question is listed as the same PT as the tester, viz. PT 1. If, however, a characteristic configuration of four or more attached chromosomes is found, the S. I. is listed as belonging to one of the other PT'S. Familiarity with the sizes and arrangements of the chromosomes in the configurations and further tests with certain other of our prime types (1) have made possible an accurate classification of the types which differ from PT 1.

*Prime type 1.* Throughout this investigation one race has been used as a standard in terms of which the chromosomes of *D. stramonium* and other species have been interpreted. Our Line 1 is a highly inbred race which came originally from Washington, D. C., and has been listed as PT 1. The twelve chromosomes of PT 1 may be arranged in Table 1 according to their relative sizes beginning with the largest (L) as 1·2. Thus far terminal humps have been located on eight of these twelve PT 1 chromosomes. They serve as convenient markers to identify chromosomes and particular ends

1) In the task of determining the chromosomal condition in F<sub>1</sub> hybrids, during a number of summers we have been aided by the following:—Helen Besley, Louise Buck, Margaret B. Chambers, Priscilla Chinn, Caroline A. Denton, O. J. Eigsti, Rachel Haynes, I. Hein, Ruth E. Henderson, Dorothy C. Hewitt, Lucy B. Hodges, Alfhild E. Johanson, R. H. MacKnight, Lois I. Platt, J. S. Potter, Sophia Satina, Jennie L. S. Simpson, H. E. Warmke.

of chromosomes. They are probably the equivalents of satellites or knobs which G. A. Lewitsky (5) demonstrated on several chromosomes in a study of root-tip cells of *Datura*. They are represented by superscripts at the appropriate ends of the chromosomes.

Table 1

L-1 <sup>1</sup> ·2	M- 9·10 <sup>10</sup>	m- 17·18
1 - 3·4	M-11·12 <sup>12</sup>	m- 19·20 <sup>20</sup>
1 - 5·6	M-13·14	S- <sup>21</sup> 21·22
M- 7·8 <sup>8</sup>	M-15·16 <sup>16</sup>	s- 23·24 <sup>24</sup>

*Prime type 2.* The "B" type which had been distinguished by breeding behavior is now called prime type 2. Instead of the chromosomes 1·2 and 17·18 of PT 1, it has the modified chromosomes 1·18 and 2·17. The hybrid between PT 2 and PT 1 shows ten bivalents plus a circle of four chromosomes in the following order:—2·17-17·18-18·1-1·2—.

When the hypothesis of segmental interchange was first proposed, it seemed to necessitate the abortion of half of the gametophytes. Hybrids between PT 1 and *stramonium* races of PT 2 have good pollen. The configuration rarely occurs as a flat circle on the spindle of the first meiotic division. Since alternate chromosomes have become attached to fibers leading to the same pole, adjacent ones to fibers leading to opposite poles, one finds a twisted circle of four chromosomes horizontally placed across the equatorial region. This zigzag arrangement is more striking in circles of six or more chromosomes, but is clearly seen in smaller circles. This orderly separation of adjacent chromosomes to opposite poles results in the formation of viable gametophytes. *Intrase stramonium* hybrids rarely show this circle broken.

The question was raised (4) whether all of the PT 2 races were really the same. By our methods only the ends of chromosomes are identified; ordinarily we cannot locate the region in the chromosome at which the interchange took place. In 1930 an examination was made of crosses between the first PT 2 race, which came from a commercial firm in Erfurt, Germany, and fourteen other PT 2 races selected from various parts of the world. One each was selected from North Carolina (U.S.A.); Chile (a purple race); Australia; Madeira Islands, French Somaliland, Pretoria and Johannesburg in South Africa; Kashmir, India; Tashkent, Asia; Portugal; Hungary; two from Italy and one from a Botanical Garden in Brazil. In all these fourteen hybrids the 1·18 and 2·17 chromosomes formed equal bivalents, and these PT 2 races therefore may be considered identical.

*Prime type 3.* Instead of the chromosomes 11·12 and 21·22 of PT 1, prime type 3 has the modified chromosomes 11·21 and 12·22. The hybrid with PT 1 shows ten bivalents plus a circle of four chromosomes in the following order:—22·21-21·11-11·12-12·22—. The

circle formation is found only rarely. Failure of attachment at the two ·22 ends produces a chain of four; failure of attachment also at the ·11 ends produces two open unequal pairs, 11·21–21·22 and 11·12–12·22. Since the members of each pair separate independently of the other, chromosomes which would be adjacent in a circle formation may go to the same pole, resulting in deficiencies and hence in inviable gametophytes. This chromosomal behavior probably explains in part why these hybrids show a variable amount of aborted pollen. That the environment has some effect on the amount of abortion is indicated by the fact that when the plants are grown in the field they regularly show about 25 per cent of aborted grains whereas this amount varies in greenhouse plants.

*Prime type 4.* Instead of the chromosomes 3·4 and 21·22 of PT 1, prime type 4 has the modified chromosomes 3·21 and 4·22. The hybrid with PT 1 shows ten bivalents plus a circle of four chromosomes in the following order:—22·21–21·3–3·4–4·22—. Failure of attachment at the ·22 ends produces a chain of four. Unlike the condition found in the PT 3 hybrids, the configuration in this PT 4 hybrid seldom is resolved into open pairs with resultant deficiencies and bad pollen. Prime type 4 hybrids, therefore, have good pollen.

*Prime type 7.* The modified chromosomes in prime type 7 have been formed by the interchange of humps rather than of larger segments of the chromosomes. Thus instead of the 9·10<sup>10</sup> and 19·20<sup>20</sup> chromosomes of PT 1, those of PT 7 are 9·10<sup>20</sup> and 19·20<sup>10</sup> in which the superscripts represent the humps. The hybrid with PT 1 shows ten bivalents plus a "necktie". The latter consists of two bivalents which are connected at the hump region only and is represented as follows:

$$9 \cdot 10^{10-10} 20 \cdot 19$$
$$9 \cdot 10^{20-20} 20 \cdot 19$$

The hybrid with PT 1 shows 50 per cent of pollen abortion. This is true also for other PT's which show a "necktie" configuration in hybrids. Since the members of each bivalent may become oriented on the spindle independently of the arrangement in the other bivalent comprising the "necktie", inviable combinations are to be expected in half of the cases, as explained in a previous paper (1).

In races which were tested several years ago, PT 7 was identified mostly by means of pollen tests. The routine then was as follows: each new race was crossed to both PT 1 and PT 7. If the hybrid with PT 1 showed 50 percent of aborted pollen grains and the hybrid with PT 7 showed good pollen, the race was classified as belonging to the PT 7 group. The "necktie" configuration of four chromosomes is frequently missing and is easily overlooked. This was particularly

true in the earlier examinations. In the more recent tests, PT 7 has been identified both by the pollen and by cytology.

*Prime type 87.* In addition to the prime types discussed in earlier paragraphs, the following two have been found each from only a single locality. Prime type 87 was obtained from Oaxaca, Mexico, from a plant which was also PT 2. It has the chromosomes 3·12, 4·22 and 11·21 instead of the 3·4, 11·12 and 21·22 chromosomes of PT 1, and in consequence the hybrid with the latter shows a circle of six in the following order: —22·4-4·3-3·12-12·11-11·21-21·22—. The chromosomes in this circle may be only partially arranged in zig-zag fashion on the spindle; or, because of weak attachments at the ·11 and ·22 ends, these six chromosomes may be arranged as a chain of six or a chain of four plus an open unequal pair. Consequently chromosomes which would lie adjacent to one another in a circle formation may go to the same pole and hence result in deficiencies and inviable gametophytes. The hybrid with PT 1 shows abortion of upwards of half of the pollen grains. Because it has the 11·21 chromosome in common with PT 3 and the 4·22 chromosome in common with PT 4, PT 87 may be the result of segmental interchange between the 12·22 chromosome of PT 3 and the 3·21 chromosome of PT 4, although as yet we have not found any PT 4 races in Central America to hybridize with the PT 3 races there.

*Prime type 88.* A second isolated prime type is PT 88 which was obtained from Entrerios, Argentina. It has the modified chromosomes 15·23 and 16·24 instead of the 15·16 and 23·24 chromosomes of PT 1. The hybrid with PT 1 shows a circle of four chromosomes as follows:—15·16-16·24-24·23-23·15—. Like PT 2, the hybrid with PT 1 shows a circle that rarely is broken. Practically no pollen is aborted.

Among the prime types secured from radiation treatment, a considerable number were produced by simple translocations in which there was a free fragment and a translocated portion of the chromosome. The hybrid of such prime types with PT 1 shows an open chain, never a closed circle. All the prime types so far found in nature were the products of segmental interchange. Except for PT 87 which was found only once, these interchanges involve only two non-homologous chromosomes.<sup>1)</sup>

1) In Table 1 of our earlier paper (4) two configurations of six were recorded in the hybrid with PT 1. One race was from Concepcion, Chile, and the other from Kashmir, India. In both cases the records show the occurrence of configurations of six in some pollen mother cells and configurations of four in other cells on the same slide. We are not prepared to offer an explanation of these discrepancies in our early records. The two S. I. races were retested and found to react only as normal PT 2.



It might be considered surprising that so few prime types have been obtained from a rather wide sampling in view of their frequency after treatments. There are only five which have been found more than once. Interchanges must have occurred several times in our cultures from untreated parents. Thus the tertiary  $2n + 4.6$  has appeared spontaneously twice, the  $2n + 13.18$  also twice and the  $2n + 2.9$  once. In these five instances the tertiary chromosomes are evidently formed by segmental interchange from normal PT 1 chromosomes. However, attempts in these cases to obtain the complementary tertiary chromosomes in homo- or heterozygous prime types have been unsuccessful. In addition, tests were made to see if, in carrying on our various  $2n + 1$  types through selfing for several years, segmental interchanges had not occurred in the sublines which had thus been established. Back-crosses to PT 1 in all of 24 cases showed only 12 bivalents and indicated that these 24 inbred strains were free from prime types induced by segmental interchange. We have no proof therefore that PT's have established themselves in our cultures except through previous treatment.

In Table 2 are listed 583 races with their geographical origin and the prime types which they represent. They are also separated into those with purple (P) flowers and those with white (W) flowers. A summary is given in Table 3. It will be noted that the sum of the number of races under the PT's is considerably more than the number of races in the second column. This is due in part to the fact that a single race may show more than one independent configuration in the hybrid with PT 1 and thus be credited to more than a single PT in the columns to the right. It is due also in part to the fact that some of the races are listed as both P and W. In Table 4 are listed those loci in which more than a single PT was found either in different individuals or associated in the same plant. In 14 loci more than a single PT was found in different individuals in the same locus. In 121 races associations of two PT's in the same plant were observed. All the races of PT 3 in nature are also PT 2, but we have isolated a pure race of PT 3 in our cultures. The maximum number of PT's associated in a single plant is three and such a condition has been observed twice.

*Datura stramonium* is widely distributed throughout the world in the semitropics and in the warmer portions of temperate regions. The location of 583 races is shown in Table 2 and on maps in figs. 1-6. In the table, races have been grouped first according to the geographical unit to which they belong and secondly under this, to the political subdivisions of this unit. Almost two-fifths of the collection came from the United States; so large a number has not been obtained

Table 2. Distribution of chromosomal prime types among 583 races of *Datura stramonium*. P—purple flower color; W—white

No. Loc	Races		Origin	PT 1		PT 2		PT 3	PT 4		PT 7		Other PT's
	Total	P W		Total	P W	Total	P W	Total	P W	Total	P W		
1	1	1 0	CANADA	1	1 0								
155	*216	171 46	UNITED STATES	*162	134 29	24 16 8			32 27 5	15 10 5			
9	21	21 0	Massachusetts	15	15 0	2 2 0				5 5 0			
6	10	10 0	Connecticut	10	10 0								
12	15	6 9	New York	14	6 8					1 0 1			
2	4	2 2	New Jersey							4 2 2			
11	13	12 1	Pennsylvania	11	11 0	2 1 1				1 0 1			
7	9	9 0	Delaware	7	7 0				2 2 0				
15	23	16 7	Maryland	19	15 4	3 0 3			1 1 0				
48	65	55 10	Virginia	36	32 4	14 11 3			23 19 4	3 3 0			
14	17	16 1	North Carolina	11	11 0	3 2 1			6 5 1	1 0 1			
1	2	1 1	District Columbia	2	1 1								
3	3	3 0	West Virginia	3	3 0								
1	2	2 0	Alabama	2	2 0								
1	1	1 0	Florida	1	1 0								
1	1	1 0	Tennessee	1	1 0								
3	3	2 1	Kentucky	3	2 1								
4	7	6 1	Indiana	7	6 1								
2	2	2 0	Illinois	2	2 0								
4	*6	5 2	Missouri	*6	5 2								
2	2	0 2	Arkansas	2	0 2								
3	3	1 2	Kansas	3	1 2								
2	2	0 2	Michigan	2	0 2								
1	1	1 0	Texas	1	1 0								
1	1	1 0	New Mexico	1	1 0								
2	3	1 2	California	3	1 2								
11	16	6 10	WEST INDIES	6	1 5	5 1 4			4 4 0	5 4 1			
1	1	1 0	Puerto Rico	1	1 0								
2	4	0 4	Virgin Islands	4	0 4								
5	8	5 3	Barbados			4 1 3			4 4 0	4 4 0			
1	1	0 1	Montserrat			1 0 1							
1	1	0 1	Haiti	1	0 1								
1	1	0 1	Cuba							1 0 1			
7	10	8 2	CENTRAL AMERICA			10 8 2		3					1 (P)
5	5	3 2	Mexico			5 3 2		2					1 (P)
1	4	4 0	San Salvador			4 4 0							
1	1	1 0	Guatemala			1 1 0		1					
58	81	56 25	SOUTH AMERICA	15	0 15	65 56 9		51					1 (W)
4	4	4 0	Colombia			4 4 0							
1	1	1 0	Venezuela			1 1 0							
14	15	0 15	Brazil	15	0 15								
1	1	0 1	Argentina										1 (W)
18	32	32 0	Peru (purple)			32 32 0		32					
5	9	0 9	Chile (white)			9 0 9							
15	19	19 0	„ (purple)			19 19 0		19					
82	*140	9 132	EUROPE	8	2 6	125 3 122		1	*23 4 20	8 2 6			
1	1	1 0	England			1 1 0							
1	3	0 3	Germany			3 0 3							
2	5	0 5	Switzerland			5 0 5							
4	6	1 5	France	4	1 3	2 0 2			1 0 1				
18	25	4 21	Spain			21 2 19		1	14 2 12	7 2 5			
8	*8	3 6	Portugal	2	1 1	3 0 3			*5 2 4				
22	36	0 36	Italy	1	0 1	35 0 35			3 0 3	1 0 1			
10	37	0 37	Hungary			37 0 37							
16	19	0 19	Russia	1	0 1	18 0 18							
23	33	5 28	ASIA	7	5 2	26 0 26							
5	7	5 2	Japan and Korea	7	5 2								

Table 2 (Continued)

No. Loci	Races			Origin	PT 1		PT 2		PT 3	PT 4		PT 7		Other PT's
	Total	P	W		Total	P	Total	P		Total	P	Total	P	
6	7	0	7	Java			7	0	7					
2	7	0	7	Siberia and Turkestan			7	0	7					
5	7	0	7	China			7	0	7					
4	4	0	4	India and Ceylon			4	0	4					
1	1	0	1	Mesopotamia			1	0	1					
18	†26	9	19	AFRICA	3	0	3	12	1	11	†12	9	5	†12 8 6
2	2	1	1	Madeira Islands			1	0	1		1	1	0	1 1 0
4	4	0	4	Algeria			4	0	4					
1	1	0	1	Liberia			1	0	1					
3	3	0	3	Portuguese W. Africa	3	0	3							
2	2	0	2	Belgian Congo			2	0	2					
1	1	0	1	French Somaliland			1	0	1					
1	1	0	1	British E. Africa			1	0	1					
4	†12	8	6	South Africa			2	1	1	†11	8	5	†11 7 6	
5	5	2	3	AUSTRALIA	1	0	1	2	0	2	2	2	0	2 2 0
2	4	4	0	HAWAII						1	1	0	4 4 0	
				BOTANICAL GARDENS	†14	9	8	29	6	23	6	6	0	8 8 0
362	583	294	296		217	152	69	298	91	207	55	80	53	30 54 38 18 2

\* One race heterozygous for purple and white.

† Two races heterozygous for purple and white.

‡ Three races heterozygous for purple and white.

Table 3. Distribution of chromosomal prime types among 583 races of *Datura stramonium*. P—purple flower color; W—white

No. Loci	Races			Origin	PT 1		PT 2		PT 3	PT 4		PT 7		Other PT's
	Total	P	W		Total	P	Total	P		Total	P	Total	P	
1	1	1	0	CANADA	1	1	0							
155	*216	171	46	UNITED STATES	*162	134	29	24	16	8	32	27	5	15 10 5
11	16	6	10	WEST INDIES	6	1	5	5	1	4	4	4	0	5 4 1
7	10	8	2	CENTRAL AMERICA			10	8	2	3				1(P)
58	81	56	25	SOUTH AMERICA	15	0	15	61	56	9	51			1(W)
82	*140	9	132	EUROPE	8	2	6	125	3	122	1	*23	4	20 8 2 6
23	33	5	28	ASIA	7	5	2	26	0	26				
18	†26	9	19	AFRICA	3	0	3	12	1	11	†12	9	5	†12 8 6
5	5	2	3	AUSTRALIA	1	0	1	2	0	2	2	2	0	2 2 0
2	4	4	0	HAWAII						1	1	0	4 4 0	
				BOTANICAL GARDENS	†14	9	8	29	6	23	6	6	0	8 8 0
362	583	294	296		217	152	69	298	91	207	55	80	53	30 54 38 18 2

\* One race heterozygous for purple and white.

† Two races heterozygous for purple and white.

‡ Three races heterozygous for purple and white.

from any other country. Races which were obtained from Botanical Gardens or Commercial seed houses are listed in the table under Botanical Gardens apart from other races because one cannot be

certain of their origin. For this reason they have not been indicated on the maps.

Prime type 1 is the predominant type in the United States; in fact, it is the only type that has been found west of the Atlantic coast. It is the only type which we have obtained from Brazil and Japan. It occurs also on some of the West Indian islands; in several countries of Europe, particularly France; in Portuguese West Africa and Australia.

If totals only are considered, the widespread distribution of PT 2 is somewhat masked by the fact that PT 1 is the predominant type in the United States from which so large a proportion of our races have come. PT 2 is the predominant type in Central America, Peru and Chile, Europe, Asia and Africa.

Prime type 3 associated with PT 2 is the only type found in Peru where all the plants observed had purple flowers. In Chile, where both purple-flowered and white-flowered races occur, the purples were PT 3 and 2 while the whites were PT 2 only. PT 3 associated with PT 2 was also obtained in Central America and Spain.

Prime type 4 is more widespread in its distribution. It is common in Virginia and North Carolina and has been found also in Maryland and Delaware in the United States; in the Barbados in the West Indies; in southern Europe, especially Spain and Portugal; in South Africa; Australia and the Hawaiian Islands.

Prime type 7 is a predominant type in South Africa and the Hawaiian Islands. It has been found along the Atlantic coast of the United States, in the Barbados, Spain, Italy, the Madeira Islands and Australia.

On the maps are indicated the loci from which races were obtained. Compound symbols made up of two or more single symbols indicate the association of more than one PT in the same plant. Numerals adjacent to a symbol indicate the number of loci in which the type was obtained in the area covered by the symbol. In each of twelve loci, two separate symbols had to be used because in these loci were found separate plants which belonged to two different PT's or PT combinations as shown in Table 4. In two loci three separate symbols were used because in each there were separate plants which belonged to three different PT's or PT associations. Not indicated on the maps are four loci beyond the limits of the part of the map of the United States shown in fig. 1 and two loci in the Hawaiian Islands.

Fig. 1 shows that PT 1 is the predominant type in the United States. Prime type 4 centers in Virginia and North Carolina where it may occur associated with PT 2 or PT 7 or with both. There is

Table 4. Loci in which more than one prime type is represented.  
P—purple flower color; W—white

Locality		Plants with more than one PT				Plants with one PT			
		No.	P	W	PT's	No.	P	W	PT's
Massachusetts	Tarpaulin Cove	1	1		2,7	3	3		1
	Barnstable					1	1		2
Pennsylvania	Spring Mills	1		1	2,7	1	1		1
Delaware	Georgetown					1	1		4
Virginia	Partlow	1	1		4,7	1	1		1
	Toano	5	5		2,4	1	1		2
	Petersburg	2	2		2,4	2	2		4
	Gardiner's Cross Roads I	1	1		4,7	1	1	1	7
	Gardiner's Cross Roads II					1	1		1
	Gordonsville					1	1	1	2
	Richmond	1	1		2,4	1	1		4
	Yorktown	1		1	2,4				
North Carolina	Jackson	2	2		2,4				
	Murfreesboro I	1		1	2,4,7	1	1		1
	Murfreesboro II					1	1		4
Barbados	Locus I	1	1		4,7				
	Locus II	1	1		2,7				
	Locus III	1	1		4,7	1		1	2
	Locus IV	1	1		4,7	1	1	1	2
Mexico	Santa Cruz	1	1		2,3				4
	Oaxaca	1	1		2,8,7				
	Huexotla	1	1		2,3				
Guatemala	Guatemala	1	1		2,3				
Peru (all purple)		32			2,3				
Chile (purple only)		19			2,3				
France	Perpignan	1		1	2,4				
Spain	Barcelona I	1	1		2,3				
	Barcelona II	1		1	2,4				
	San Adrian I	2		2	2,4				
	San Adrian II	3		3	2,4				
	San Adrian III	3		3	2,4				
	Montseny I	1		1	2,4				
	Montseny II	2		2	2,4				
	Palos	3		3	2,7				
	Aragon	1	1		4,7				
	Madrid	1	1		4,7				
Portugal	Lisbon I	1		1	2,4				
	Lisbon II	1		1	2,4				
Italy	Rome I	1		1	2,4,7	3		3	2
	Rome II	1		1	2,4	1		1	2
	Piedmont	1		1	2,4				
Madeira Islands		1	1		4,7				
South Africa	Pretoria	1		1	2,7				
	Johannesburg I	*8	5	4	4,7				
	Johannesburg II	*1	1	1	4,7				
	Bulawayo	1	1		4,7				
New South Wales	Gunnedal	1	1		4,7				
Queensland	Moreton Bay	1	1		4,7				
Hawaii	Molakai	1	1		4,7				
Botanical Gardens									
	Madrid, Spain	4	4		4,7				
	Rio, Brazil	1	1		2,4				
	Havana, Cuba	1	1		4,7				

\* One race heterozygous for purple and white.

a scattering of PT 2 and of PT 7 to a lesser extent along the Atlantic coast in the United States.

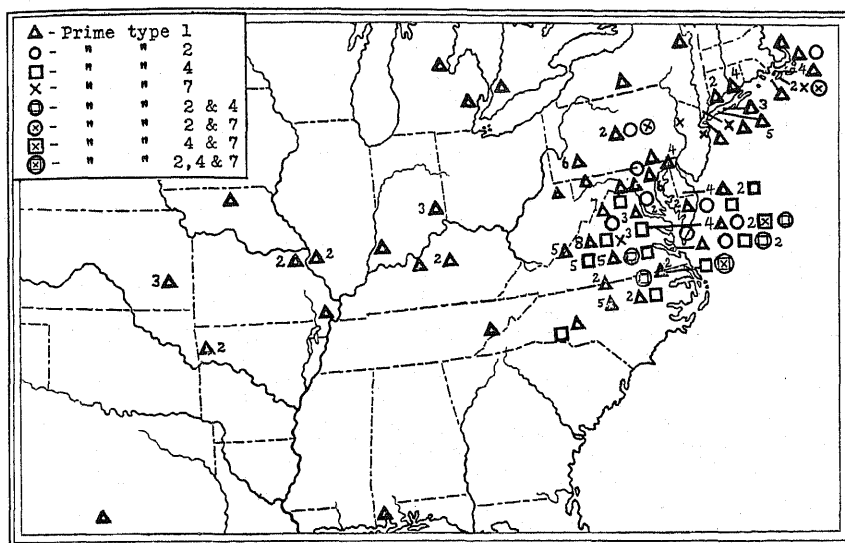


Fig. 1. Distribution of prime types in Central and Eastern United States. In this and following maps, numerals adjacent to a symbol indicate the number of loci of this type. Because of limited space in some states along the Atlantic Coast, the symbols have been drawn to the right with lines indicating the loci. Prime type 1 was obtained also at Gulfport, Florida; Haynes Canyon, New Mexico; Sacramento and Palo Alto, California.

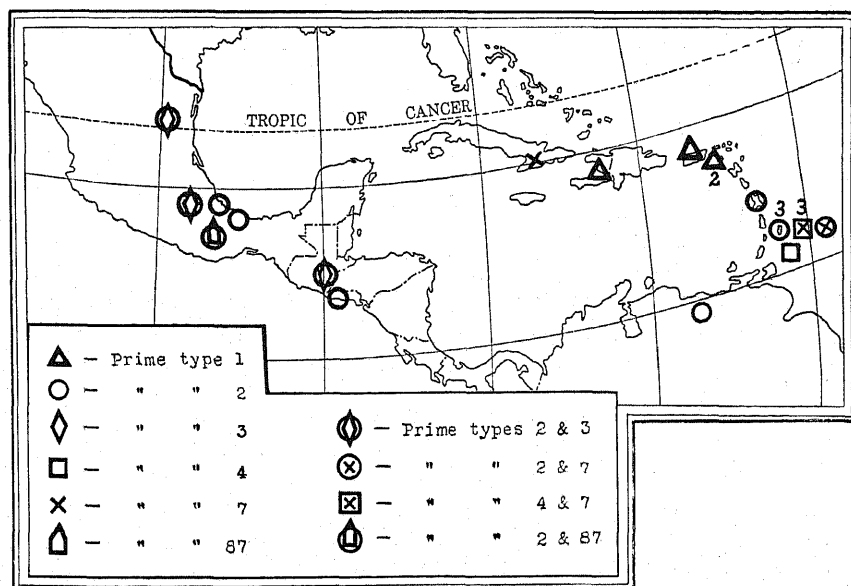


Fig. 2. Distribution of prime types in Mexico, Central America and the West Indies

In contrast, South America, as indicated in fig. 3, shows separation of types. All of the collections from Brazil belong to PT 1 type. In Chile all white plants were PT 2; all purple plants, PT 2 plus PT 3. In Peru only purple flowered plants were found, all being PT 2

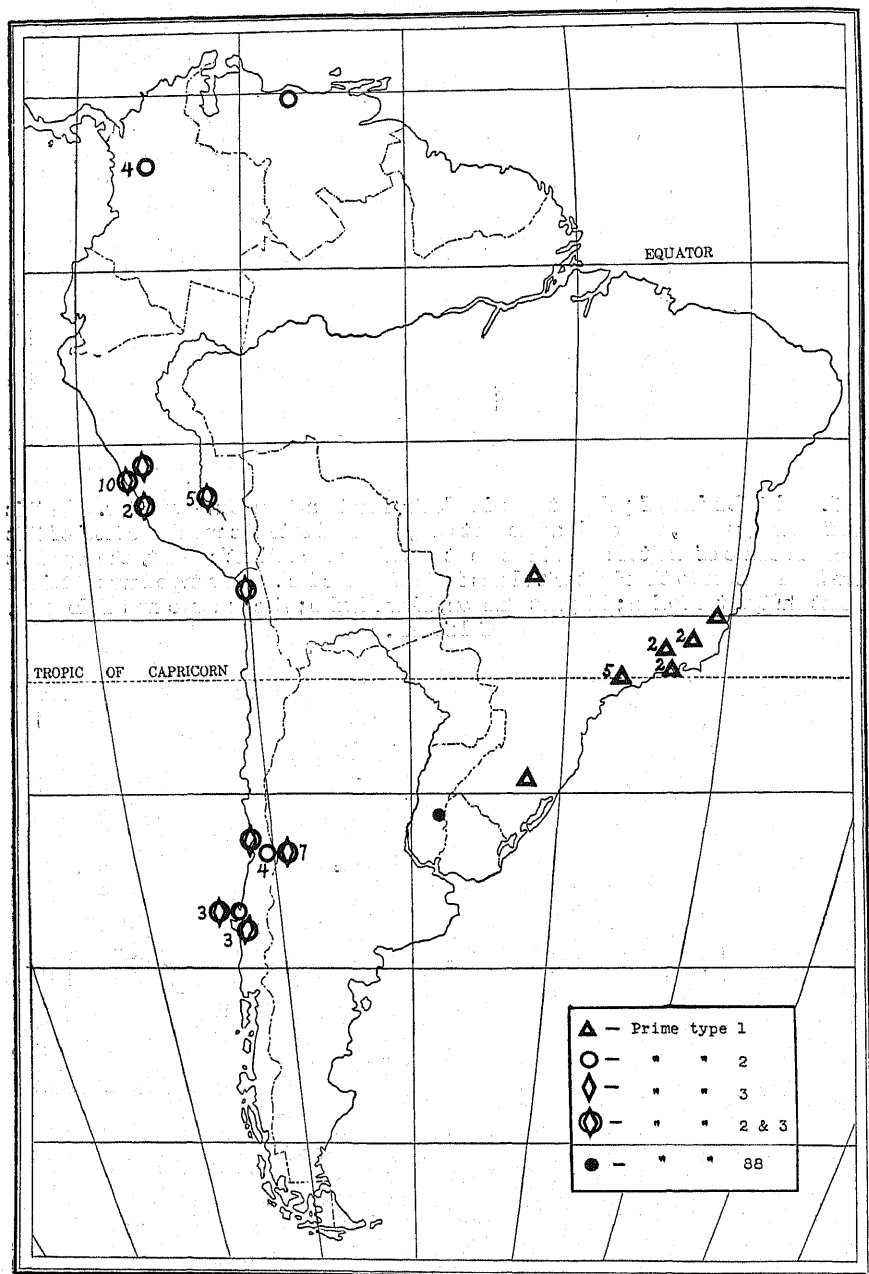


Fig. 3. Distribution of prime types in South America.



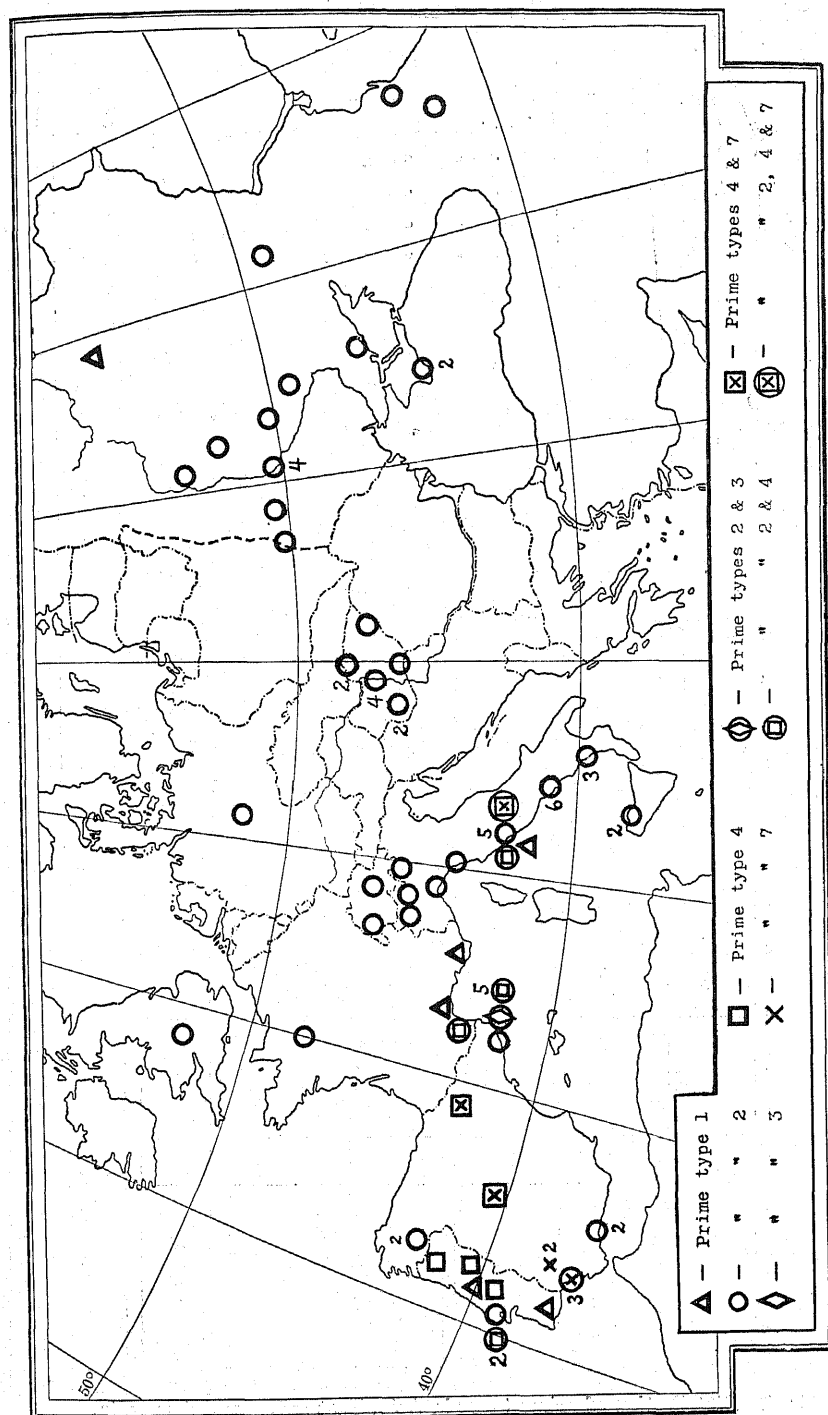


Fig. 4. Distribution of prime types in Europe.

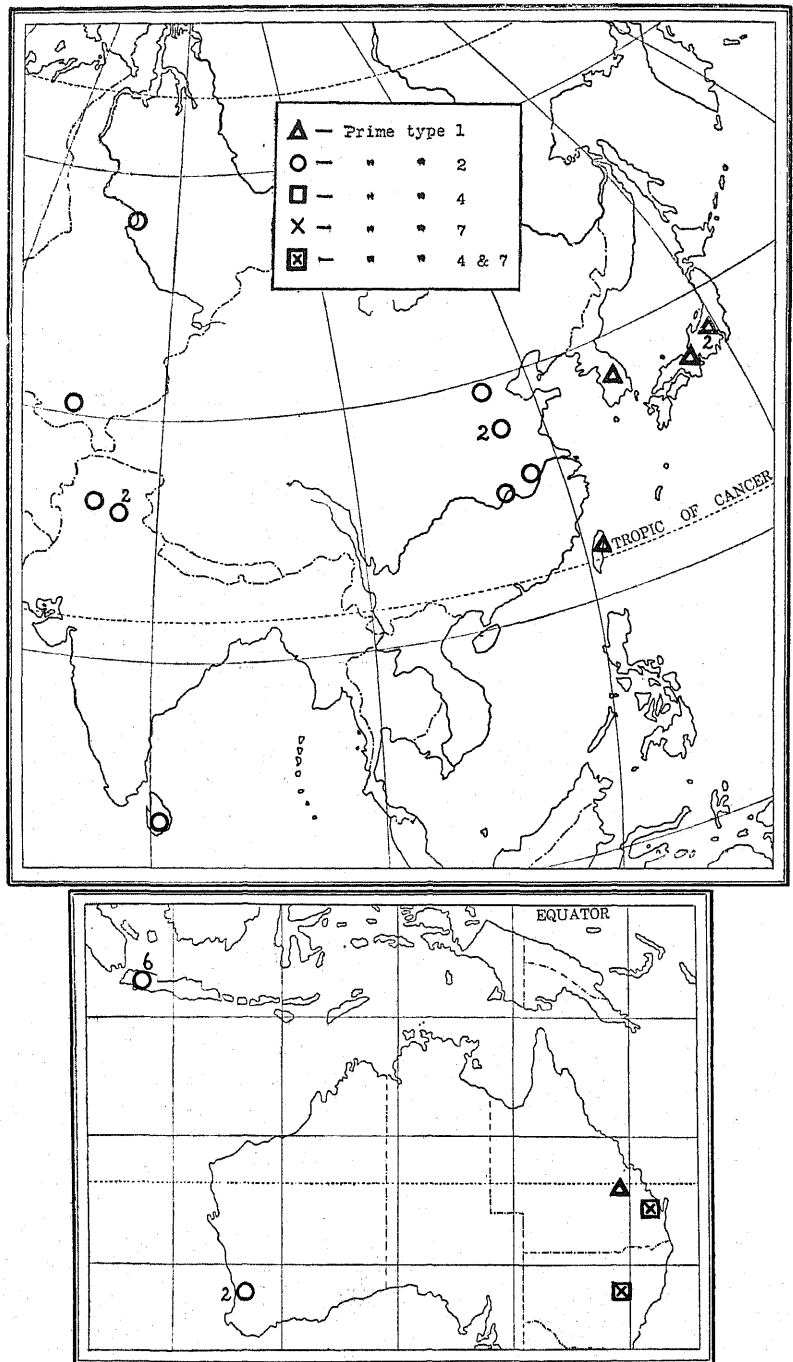


Fig. 5. Distribution of prime types in Asia, East Indies and Australia.

plus PT 3. In addition a new type (PT 88) was obtained from northern Argentina. A rather careful search for *Daturas* was made by one of us while on a collecting trip through the Argentine. From our own observations in the field, from inspection of specimens in the principal herbaria of the country and from reports of botanists whom we consulted, it is safe to say that although *D. ferox* is common, *D. stramonium* is not known to occur in the central and southern parts of this country. The collection which gave us PT 88 is the only record we have of the occurrence of *D. stramonium* in the Argentine. It is possible that further collections in the same general region might disclose the fact that PT 88 is not a sporadic prime type but is representative of the races in this part of the country.

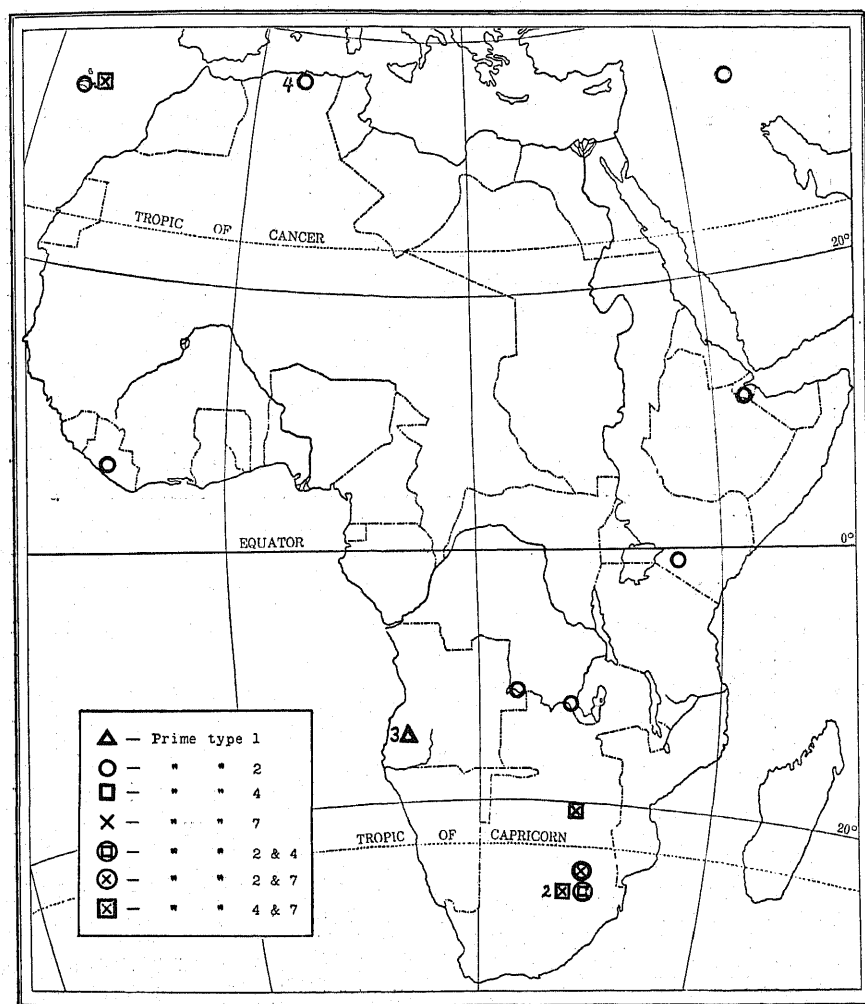


Fig. 6. Distribution of prime types in Africa and Southwestern Asia Minor.

In fig. 4 one can see that PT 2 is the predominant type in Europe; in fact it is the only type except in Portugal, Spain, southern France and western Italy and one locus in northern Russia.

The predominance of PT 2 types also on the mainland of Asia is shown in fig. 5. The condition is a continuation of that in eastern Europe. Prime type 1 occurs in Japan and Australia. In the latter, PT 4 and PT 7 also have been found.

While it is true, as shown in fig. 6, that PT 2 is widespread in most of Africa, in southern Africa PT's 4 and 7 predominate and on the west coast PT 1 has been found.

### Discussion

The preceding paragraphs have presented the data regarding the distribution of prime types in our collection of races of *D. stramonium*. Certain facts seem to stand out rather clearly. There are areas in which certain types appear to occur exclusively so far as their races have been tested. Among these are the Peruvian area which contains purple-flowered PT 3 associated with PT 2, Brazil which contains a white-flowered PT 1, Eastern Europe and Western Asia with a white PT 2, and Japan with PT 1 including both purples and whites. On the other hand there are areas in which a mixture of types is found. As examples may be mentioned the Eastern states of the United States, the Southwestern part of Europe including Southern France, Spain, Portugal and Western Italy, Central America and the West Indies, and Australia.

*Datura stramonium* is a weed and is likely to be found in disturbed soil wherever the climatic conditions are favorable. Its place of origin is unknown. Gray's *Manual* as well as Britton and Brown's *Flora* describe the two Linnean species, *D. stramonium* and *D. tatula* as distinct species although the two are merely color forms determined by a pair of genes for white and purple respectively. The purple form is listed as naturalized from tropical America and the white form as naturalized from Asia. The latter statement, however, is qualified by a question mark. Let us see if evidence from our prime types may have a bearing on this problem of the origin of the purple and white forms in the United States. Our collection of races from this country is predominantly purple (171 P: 46 W) and is predominantly PT 1 (162 PT 1; 24 PT 2; no PT 3; 32 PT 4; 15 PT 7). In South America the only known source of PT 1 is in Brazil, but all the plants of which we have records from Brazil are whites. Whites are distinctly in the minority in the United States. Among the races from Central America, purples predominate but they are all PT 2's. PT 2 is rare in the United States. The West Indies have all the types which occur

in the United States although our collections from there show a relatively smaller proportion of PT 1's and the latter differ from those in the United States by being mostly whites. If we look outside this country for a place of origin of the predominant purple PT 1's which occur here, the only place which our records suggest is Japan, but the data from that country so far are rather meager.

We have made tabulations of the proportions of purples and whites among the different prime types as shown in Table 5. In states where PT 2 occurs, whites have been found four times as frequently in PT 2 as in PT 1, a fact which suggests that if PT 2 was introduced into this country, it was brought in chiefly in the white-flowered form. If we seek for such a source of supply, it would be natural to look to Europe where white PT 2's predominate or perhaps to Africa whence they might have been brought in with the slave trade. A glance at Table 2 and the map in fig. 1 will show that in the United States, PT 2, PT 4 and PT 7 are confined to the eastern states. The western states which show only PT 1's, though relatively poorly sampled, have twice as many races tested as has North Carolina, for example, which has representatives from all the PT's which occur in North America. This eastern area, therefore, may be considered the area where the different types were introduced or perhaps where they originated. From Table 5 it might appear that

Table 5. Occurrence of purples and whites in prime types in selected states in United States

5 states with PT 2 records			4 states with PT 4 records			6 states with PT 7 records		
Prime type 1			PT 2			PT 1		
PT 2			PT 4			PT 1		
PT 1			PT 7			PT 1		
Total	P	W	Total	P	W	Total	P	W
92	84	8	24	16	8	73	65	8
Ratios	10.5	1		2	1		8	1
Expectation from PT 1 records			22			28.5		

both PT 4 and PT 7 are somewhat more likely to be whites than purples. The tendencies at best are slight and the numbers are not large. It is doubtful, therefore, if conclusions can be drawn safely from these facts which would favor PT's 4 and 7 also being introduced in white plants.

Frequently collections from a single patch of plants (locus) disclosed more than a single PT or color form. Such cases are summarized in Table 4. They include cases in which a single plant had to be recorded as belonging to two or three PT's. In other cases different PT's were represented in separate individuals in the same locus. The association of PT's together in the eastern United States

is summarized in Table 6. Prime type 7 occurs associated with PT 2 about as frequently as one would expect from the proportion of PT 2 races tested for the region in question. Prime type 4, however,

Table 6. Occurrence of prime type 2 with PT 4 and PT 7 in United States

4 states with PT 4 records						6 states with PT 7 records					
Not prime type 4			Prime type 4			Not prime type 7			Prime type 7		
Total	Not PT2	PT2	Total	Not PT2	PT2	Total	Not PT2	PT2	Total	Not PT2	PT2
82	74	8	32	20	12	120	102	18	15	12	3
Ratios	9.25	1		1.66	1		5.6	1		4	1
Expectation from non-PT 4 records				29	3	Expectation from non-PT 7 records				12.7	2.3

appears to be associated with PT 2 about four times as often as one would expect. The same reasoning employed in discussing the association of white flower forms with PT 2 would suggest that PT 4 came into the United States in association with PT 2. This conclusion could not be extended to all other countries since it will be observed from Tables 2 and 3 that PT 4 is predominantly purple in Africa and exclusively purple in Australia and in Botanic Gardens.

Prime type 4 and PT 7 are seen from Table 4 to be frequently associated together. Out of 32 occurrences of PT 4 in the United States, three were associated with PT 7. In other parts of the world, however, they are associated with one another more frequently. Thus out of 80 PT 4's, 28 were associated with PT 7. In terms of PT 7 the frequency of association is still more striking. There are 54 PT 7's in our collection. Of these, 28 or more than half were found associated in the same plants with PT 4. These facts suggest that PT 7 may have originated in a PT 4 race and have been separated through hybridization in cases in which it occurs alone. Similarly, since PT 2 is widespread and PT 3 is restricted in its range but is always associated with PT 2, it may be concluded that PT 3 arose in a PT 2 race.

We have discussed the origin and distribution of types in terms of PT 1 as a standard. A standard we believe is essential, but the choice of PT 1 was accidental, due to its predominance in this country. We could use any standard type without implying thereby that this race was phylogenetically the origin of other races in this and other species. With our present knowledge, we should now choose PT 2 in all probability. Prime type 2 appears to be more widely distributed. It also shows a closer relationship with other species tested. Thus the standard tester races of *D. ferox*, *D. quercifolia*, *D. Leichhardtii* and probably *D. pruinosa* contain the PT 2 modified

chromosomes 1·18 and 2·17. The tester race of *D. discolor* has the PT 2 chromosome 2·17 but the 1·18 chromosome has been modified by further interchange. If one were to use any single PT of *D. stramonium* as the primitive form from which to derive all other species of the genus, it would appear natural, therefore, to choose PT 2.

Another PT which shows relationships with other, though fewer, species is PT 3. The PT 3 chromosomes 11·21 and 12·22 occur in the tester races of *D. ferox* and *D. quercifolia*, which with *D. stramonium* make up the *stramonium* group. Furthermore, the tester race of *D. discolor* is a modified PT 3. If one were to choose a race from Peru as a standard, one would have PT 2 associated with PT 3. In consequence hybrids between it and the two other members of the *stramonium* group would show fewer configurations than if any other known race of *D. stramonium* were used as a standard. It is perhaps significant that the distribution of the two other members of the *stramonium* group overlaps that of PT 3. *D. quercifolia* occurs in Central America where PT 3 and PT 2 also occur associated together, and *D. ferox* is abundant as the only *Datura* species common in central Argentina and spreads over into Chile where the purple *D. stramonium* plants are PT 3 associated with PT 2.

One of us in 1925 discovered hybrids between purple *D. stramonium* and *D. ferox* growing in great abundance in the extensive vineyard of the "Quinta Normal" in Santiago, Chile. We cannot be sure of the direction of the stream of evolution and hence dare not say that *D. ferox* and *D. quercifolia* were derived from the "Peruvian" PT 2-PT 3 type or that the "Peruvian" type of *D. stramonium* was derived from one of these other species. We can say with confidence, however, that their chromosomes are related. The Peruvian type is therefore of especial interest. All the PT 3 races from Peru and Chile are similar in appearance with a characteristic lobing of the leaves and with long spines on the capsules. The one race from Spain is the same in appearance and suggests a migration to or from this mother country. The three races in Central America resemble less closely the plants from Peru.

Prime types 4 and 7 are widely scattered. They differ from PT's 1, 2 and 3 in that in no great area do they appear to be present to the exclusion of other PT's. Their characteristic chromosomes have not been found in any of the other species tested. Prime types 4 and 7 therefore may be listed as probably peculiar to *D. stramonium*. There is no evidence that they have arisen in other species.

We have discussed the distribution of PT's as if the interchange of chromosomal segments to produce each PT had occurred but once



as a mutation in a single plant, and the PT race thus formed had been distributed to other regions. Such an interpretation is supported by the *intrase* crosses between PT 2 races from widely separated regions already mentioned. They indicated that the modified chromosomes in these cases were alike so far as the interchanged segments were concerned. The relatively few PT's in nature would also suggest the extreme rarity of the segmental interchanges necessary to their formation. In our collection of PT's induced by treat-

Table 7. Chromosomes involved in prime types

PT 1 chromo- somes	Times modified to from PT's		
	Follow- ing treat- ment	From nature	
		Recurrent types	Isolated types
1-2	18	1	
3-4	12	1	1
5-6	11		
7-8	14		
9-10	17	1	
11-12	17	1	1
13-14	19		
15-16	18		1
17-18	8	1	
19-20	13	1	
21-22	10	2	1
23-24	12		1

ment, there are 82 which are due to segmental interchanges or simple translocation. As shown in Table 7, the modified chromosomes in these PT's are well scattered among the 12 PT 1 chromosomes. There are several instances in which the PT's involve the same chromosome ends. In only one instance, however, could the two PT's possibly be identical in respect to the place where the interchange occurred. Further study might show that in this case also the two PT's were not actually the same. We can be sure that duplication of segmental interchanges following treatment at least is extremely rare. There are lines of evidence on the other hand which indicate that breaks in chromosomes may occur repeatedly at the same place. It has already been shown that two of the three different tertiary  $2n + 1$  types which have appeared spontaneously in our cultures have appeared again independently. These tertiary forms have an extra chromosome formed by segmental interchange, and in these two repeated types the evidence seems good that the interchanges took place at the same level of the chromosomes. Moreover in our secondaries, such as the  $2n + 2:2$  type (in which the extra chromosome appears to be formed by a break in two homologous chromosomes and the reattachment of similar parts to form double half chromosomes) the breaks in the chromosomes appear to be at the same place in all the cases in which the same chromosomes are affected. There is evidence from our experience, therefore, indicating that there are preferred places in chromosomes at which breaks repeatedly take place. These facts offer some presumption in favor of the same segmental interchange having occurred independently in different regions. However, there

is no direct evidence in favor of the polyphyletic origin of prime types in nature.

The modified chromosomes in prime types we have interpreted as resulting from segmental interchange between non-homologous chromosomes of our standard PT 1. Our method of testing, however, detects only the changed ends. It is likely that chromosomal changes have occurred inside the chromosomes of prime types in addition to gene mutations which are known to have occurred in some cases and which may differentiate geographical races. It is not believed that such possible internal chromosomal changes or gene mutations would have any necessary relation to the type of interchange responsible for the prime types in question. This conclusion is reached from a study of tertiary  $2n + 1$  types derived from prime types. All the recurrent prime types have repeatedly been backcrossed to PT 1 with retention of the hybrid chromosome configuration until the plants were indistinguishable in appearance from our standard PT 1. Ultimately the prime type is then extracted in homozygous condition free from any genes which may have been peculiar to the race from which the PT was originally obtained. By the proper breeding procedure each of the modified chromosomes may be made the extra in a  $2n + 1$  tertiary type in which all the chromosomes except this extra belong to PT 1. Thus from PT 2 we have isolated the two tertiaries  $2n + 1:18$  and  $2n + 2:17$ . By this means we have isolated the modified chromosomes as extras in  $2n + 1$  forms from all the recurrent prime types. We have in these cases apparently replaced all the unmodified chromosomes by PT 1 chromosomes and through crossing over have likely also replaced a large proportion of the modified chromosomes by parts of the PT 1 chromosomes from which they had been derived. Any recessive genes remaining would certainly be suppressed by the dominant allelomorphs in the unmodified chromosomes. The appearance of these purified  $2n + 1$  tertiaries derived from the prime types is what would be expected on the supposition that segmental interchange alone were responsible for their formation.

It is noteworthy that no simple translocations have been observed in nature in *Datura*. They have occurred not infrequently, however, following radiation treatment. They would have given a means of adding extra chromosomal material and thus changing the chromosomal balance with a wide range of effects upon the structure of the plant affected. Thus from the radium-induced prime type in which the .2 half of the 1·2 chromosome had been translocated to the 11·12 chromosome with the .1 half left free, we have eliminated the free fragment and synthesized a pure-breeding type (6) homozygous for

the 2·11·12 chromosome which differed in many characteristics from the normal on account of the extra chromosomal material which it contained. We have developed a method (7) whereby it is possible to synthesize pure-breeding types through the addition of internal excess material. This involves the combination of induced prime types in which the segmental interchange occurred between the same chromosomes but at different levels. The fewness of prime types of *Datura* in nature renders this particular method of adding chromosomal material internally unlikely in our genus. We are still of the opinion earlier expressed (8) that the addition of excess chromosomal material would furnish the kind of differences observed between species and that in some way such addition of blocks of genes has been operative in evolution. We must confess, however, that the present study presents no evidence in support of this belief. Our contribution refers to changes in the end arrangements of chromosomes. Though similar changes have occurred within all the species of *Datura* adequately tested and have at least accompanied the formation of new species, we do not yet know their significance in evolution. Our study at least presents a body of facts regarding what has actually taken place in nature in the vital units responsible for variation in the organic world. The relation of these units to the origin of species is a field which has been sadly neglected. We feel it cannot be too strongly emphasized that the clue to the evolution of species lies in a study of the evolution of their chromosomes.

### Summary

Data are presented on the distribution of chromosomal prime types (PT's) in 583 races (287 purples, 289 whites and 7 races which were heterozygous for P and W) from 362 loci exclusive of 51 races from Botanic Gardens and commercial firms. Prime types found in nature consist exclusively of races produced by segmental interchange between non-homologous chromosomes in terms of a standard race, PT 1. With the exception of two single races, only five prime types (PT's 1, 2, 3, 4, 7) have appeared in our collections. Some areas are represented by a single PT or PT association. Thus, all the races from Eastern Europe with the exception of one race from Russia and all the races from Asia with the exception of Japan are white-flowered PT 2; all the races from Brazil are PT 1 and also have white flowers; all the races from Peru are PT 3 associated with PT 2. In some areas a mixture of types has been found, for example, in South-western Europe, the West Indies and Central America. In the United States most of the races are purples and predominantly PT 1 with PT's 2, 4 and 7 occurring scattered within the eastern area. Whites

are found more frequently among the PT 2's than among the PT 1's, a fact which suggests that PT 2 was introduced into this region as a white race from Europe or Africa where white PT 2's are predominant. Prime type 3 has always been found associated with PT 2 which latter PT is the one most widely distributed. It is concluded that PT 3 probably arose in a PT 2 race. The two other species of the *stramonium* group, *D. quercifolia* and *D. ferox*, have been found within the range of the "Peruvian" PT 2-PT 3 race. Since the tester races of these two species both have the four chromosomes of PT 2 and PT 3, it is suggested that these two species are phylogenetically related to *D. stramonium* through PT 2 and 3. Prime type 2 chromosomes are found in the majority of the *Datura* species tested. Prime type 4 and PT 7, however, appear to be limited to *D. stramonium*.

#### Literature cited

- 1 Bergner, A. D., Satina, S., and Blakeslee, A. F. Prime types in *Datura*. Proc. Nat. Acad. Sci. 19: 103-115. 1933.
  - 2 Blakeslee, A. F. Variations in *Datura* due to changes in chromosome number. Amer. Nat. 56: 16-31. 1922.
  - 3 Beling, J., and Blakeslee, A. F. On the attachment of non-homologous chromosomes at the reduction division in certain 25-chromosome *Daturas*. Proc. Nat. Acad. Sci. 12: 7-11. 1926.
  - 4 Blakeslee, A. F. Cryptic types in *Datura*. Jour. Hered. 20: 177-190. 1929.
  - 5 Lewitsky, G. A. Investigation on the morphology of chromosomes. Proc. U. S. S. R. Cong. Genet. 2: 87-105. 1929.
  - 6 Blakeslee, A. F., Bergner A. D., and Avery, A. G. Methods of synthesizing pure-breeding types with predicted characters in the Jimson Weed, *Datura stramonium*. Proc. Nat. Acad. Sci. 19: 115-122. 1933.
  - 7 — A new method of synthesizing pure-breeding types with extra chromosomal material in *Datura*. Amer. Nat. 70: 255-257. 1936.
  - 8 Blakeslee, A. F. The species problem in *Datura*. Proc. 6th. Intern. Cong. Genet. 1: 104-120. 1932.
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## L'aspect et nombre des chromosomes méiotiques chez

### *Sweetia perennis*

par

Z. Wóycicki

J'ai constaté dans mon travail sur *Gentiana lutea*<sup>1)</sup> que le dessin de GUÉRIN, représentant la coupe transversale du sac pollinique de cette plante, présente de grandes différences avec le matériel sur lequel j'ai opéré. Cette coupe rappelait en même temps la structure histologique des parois des sacs polliniques de *Sweetia*. Chez cette dernière précisément la paroi des sacs polliniques est composée, dans la période plus précoce de celle, que nous voyons sur le dessin de GUÉRIN, de 5 couches<sup>2)</sup> bien distinctes. Parmi celles-ci deux externes, l'épiderme et l'hypoderme, sont composées de cellules hautes, tandis que les autres trois sont composées de cellules plates. Dans la susdite période c'est encore l'hypoderme qui présente les tendances bien nettes à la division sur toute son étendue: dans plusieurs points on peut constater de nombreuses métaphases.

A la limite de la vraie paroi de l'anthère repose, sur la face interne du sac pollinique, une couche de petites cellules qui se déchirent à la suite de la contraction de l'archéspore, pendant la fixation (fig. 1). Le tissu remplissant le sac pollinique est composé de deux catégories de cellules. Les unes sont remplies de protoplasme granuleux dans lequel repose un grand noyau, contenant un grand nucléole (et un ou quelques petits nucléoles), les autres possèdent le protoplasme transparent, muni de grandes vacuoles (fig. 1) et d'un grand noyau (renfermant un ou parfois deux nucléoles). Les unes aussi bien comme les autres sont disposées en rangs discontinus dans le même plan: ceux-ci sont interrompus au milieu de la largeur du sac pollinique.

En comparant les coupes longitudinales avec les coupes transversales on voit que le tissu stérile forme une série de mailles dans lesquelles les cellules mères du pollen traversent différentes phases, parfois assez distantes les unes des autres. Cette description harmonise entièrement avec celle de GUÉRIN (1924).<sup>3)</sup>

Dans les périodes prophasales la ligne, le long de laquelle se fait la contraction du contenu du sac pollinique, apparaît sous l'aspect

1) WÓYCICKI, Z., 1935.

2) Dans certains anthères leur nombre arrive même jusqu'à 7.

3) "Ce tissu est.....très abondant et forme une sorte de réseau....."  
L.c., p. 1621.

d'un fossé entre deux couches du tapetum, parce que chez *Sweertia* — en comparaison avec les *Gentianes*, que j'ai eu l'occasion d'étudier — le tissu "nourricier" est composé de deux assises. Il se forme ici de cette façon une sorte de couche spécifique "fondamentale" ou couche de transfusion sur laquelle sont montées des cellules tapétales qui prolifèrent vers l'intérieur du sac pollinique.

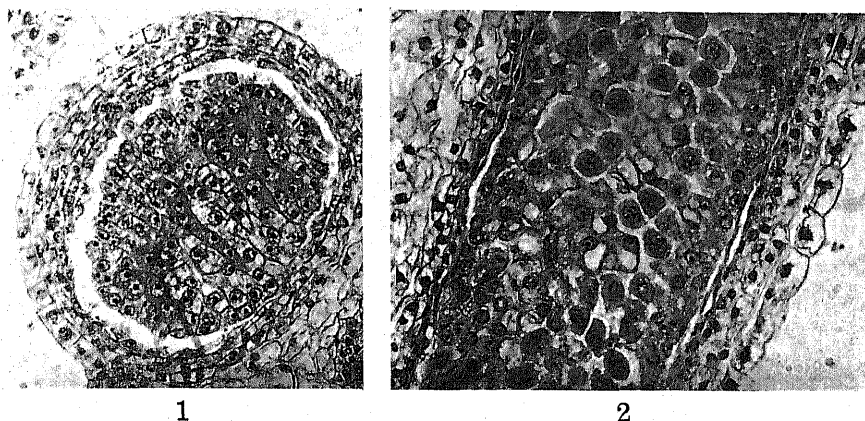


Fig. 1. *Sweertia perennis*. Coupe transversal du sac pollinique pendant la période de différenciation de l'archéspore. On voit ici la paroi de l'anthere, composée de 6 assises de cellules, le tissu nourricier qui est formé de deux assises et des rangées de cellules-mères du pollen qui alternent avec des rangées de cellules qui, suivant l'expression de GUÉRIN, "concourent à la formation du tissu nourricier".<sup>1)</sup>  $\times \pm 350$ .

Fig. 2. *Sweertia perennis*. Coupe longitudinale du sac pollinique pendant la période des prophases des cellules-mères du pollen. On voit: la paroi de l'anthere, le tissu nourricier composé de deux assises de cellules, le tissu stérile en forme de réseau dans les mailles duquel se trouvent des vraies cellules-mères du pollen. Les cellules "nourricières" se jettent immédiatement aux yeux à cause de leur forte vacuolisation.<sup>2)</sup>  $\times \pm 350$ .

Pendant la période des prophases (surtout au stade du synapsis des cellules-mères du pollen) les cellules épidermales, dans la région suturale, ainsi que les cellules situées plus profondément, se divisent activement, ce qui conduit, dans la région du crampon stomiale, à l'augmentation du nombre de cellules situées les unes à côté des autres et les unes au dessus des autres.

La formation de l'assise nourricière à deux couches de cellules a lieu de très bonne heure, car dans les sacs de la fig. 1, c'est à dire dans la période où on peut seulement distinguer de véritables

1) GUÉRIN, P., L.c., p. 1621.

2) D'après GUÉRIN "les cellules-mères définitives du pollen...qui séparent des rangées de petites cellules demeurées stériles....." L.c., p. 1621.

On constate pourtant sur la fig. 1 et 2 que les cellules stériles chez *Sweertia* ne sont pas plus petites mais au contraire elles sont dans ce moment plus grandes pour la plupart des cas que les cellules fertiles.



cellules archésporiales de cellules stériles, cette assise est déjà double. Ce phénomène (de dédoublement) est probablement en relation avec le fait que les cellules de l'assise nourricière, tournées vers l'intérieur du sac pollinique, restent uninucléaires, c'est à dire que la différenciation de la dite assise en deux couches a épuisé la possibilité de la division des noyaux de ses cellules.

Deuxième caractère, qui distingue *Sweertia* de *Gentiana*, c'est l'aspect des chromosomes. Chez *Sweertia perennis* dans la période du leptotène tardif (fig. 3), du zygotène-pachytène (fig. 4) et du diplotène précoce (fig. 5) les chromosomes possèdent une structure "chromomérique" nette.<sup>1)</sup> Sans entrer, tout au moins pour le moment, dans la discussion au sujet de la nature de la structure "chromomérique" des chromosomes, qui est, ce dernier temps, l'objet de vives discussions et de contradictions (SCHAFFSTEIN, G., 1935; HERMANS, G., 1936) je me borne en ce moment de fournir seulement quelques observations indiquant la différence qui existe dans l'aspect et sans

doute dans la constitution des chromosomes chez les *Gentianes* et chez *Sweertia*.

Au leptotène alors dans les noyaux des cellules-mères du pollen chez *Sweertia* nous avons à faire avec les filaments minces d'une structure perlée. Les filaments sont disposés à peu près parallèlement deux à deux, ou enroulés<sup>2)</sup> l'un autour de l'autre (fig. 3).

Au zygotène les chromosomes associés (fig. 4) corres-

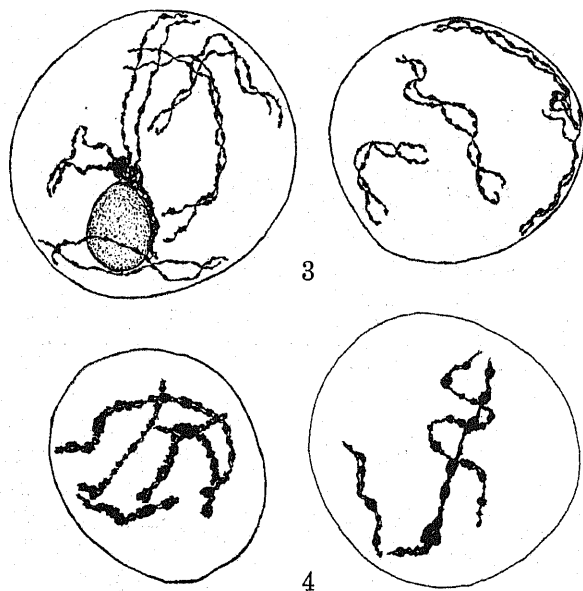


Fig. 3. *Sweertia perennis*. Deux noyaux au leptotène tardif. Les filaments minces, disposés par paire, montrent une structure "chromomérique."  $\times \pm 2500$ . Fig. 4. *Sweertia perennis*. Deux noyaux au stade zygotène. Les chromosomes homologues à la structure "chromomérique" entièrement conjugués.  $\times \pm 2500$ .

1) D'après HEITZ (1935): „Die Chromomeren sind fast ausschließlich vom Leptotän bis zum Pachytän sichtbare.....kugelige gesetzmäßig verschieden große Gebilde“. (L.c., p. 422).

2) Comme par exemple chez *Dasyurus* (P. C. KOLLER, Cytologia, V. 7, 1936).



pondent plus ou moins à ce que nous voyons au dessin 3 (page 61) de DARLINGTON (1935).<sup>1)</sup>

Au moment du pachytène on a à faire avec les chromosomes, qui forment des entrelacements et des boucles d'une structure perlée soit sur toute la distance, possible à observer au microscope, soit sur certaines tranches, donc avec les intervalles. Dans ces intervalles les chromosomes, mutuellement couplés, forment une bande aux bords effilochés qui rappelle beaucoup, par sa structure, le segment frontal (a) de la fig. 21 de DARLINGTON (1935), ce que cet auteur appelle "nearly complete pairing. ....".<sup>2)</sup> Il y a toujours dans cette période un seul nucléole qui repose au centre du noyau. Celui-ci ne présente (à la suite de l'application de notre fixateur)<sup>3)</sup> aucune structure, rappelant, tout au moins en partie, de fines structures des réseaux faiblement colorés, représentés sur la fig. 4, pl. 27 de TAYLOR (1931).

La structure "chromomérique" se maintient chez *Sweetia* encore dans le diplotène précoce (fig. 5), pendant lequel les chromosomes homologues possèdent le caractère très rapproché de la figure 24, p. 68 de DARLINGTON (1935).

Ils la perdent dans le diplotène tardif (et surtout au strepsitène) quand les chromosomes homologues

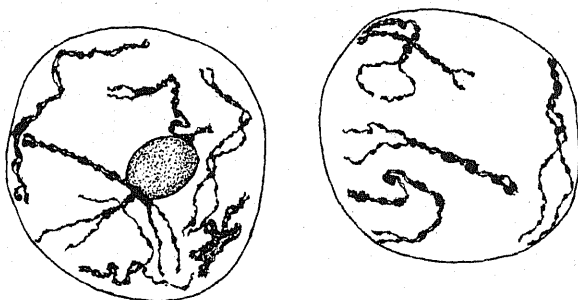


Fig. 5. *Sweetia perennis*. Deux noyaux au stade diplotène précoce. La plupart des segments montrent déjà la séparation des chromosomes homologues, qui sont partiellement entrelacés. La structure "chromomérique" des homologues séparés, encore bien conservés.  $\times \pm 2500$ .

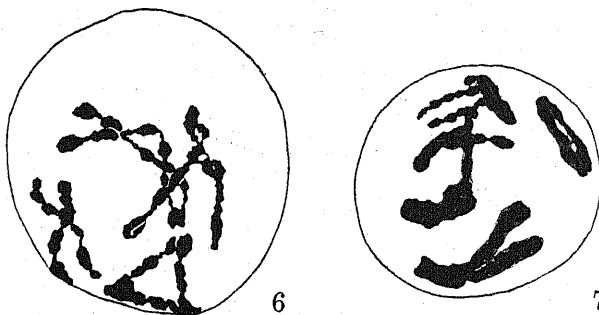


Fig. 6. *Sweetia perennis*. Le noyaux au stade diplotène-strepsitène.  $\times \pm 2500$ . Fig. 7. *Sweetia perennis*. Le noyaux au stade de diacinese précoce. Les cordons noueux, couverts par la substance peu colorable (matrix)  $\times \pm 2500$ .

1) DARLINGTON parle de "double pachytène" dans le texte et de "zygotène" dans l'explication des figures 1-6 page 61.

2) DARLINGTON, C. D., l.c., p. 67.

3) WÓYCICKI, Z., 1932.

se raccourcissent, s'épaississent et forment originellement les cordons irrégulièrement noueux (fig. 6).<sup>1)</sup>

Les chromosomes méiotiques chez *Sweetia* forment habituellement dans la diacinèse précoce (fig. 7) des couples un peu parallèles ou en forme de V, caractérisés par leur union terminale et par la présence d'une substance peu colorable (matrix).

C'est seulement certains parmi les chromosomes et surtout les plus longs qui forment, il me semble, des ciseaux ou tenailles avec les "interstitial chiasmata", qui d'après BELLING<sup>2)</sup> possèdent une importance fondamentale pour l'échange des gènes. Dans certains cas les noyaux au stade de diacinèse plus avancée renferment, chez



Fig. 8a et 8b. *Sweetia perennis*. Gemini en forme de V, des ellipses ou cercles. Certains parmi eux (fig. 8b) forment des tenailles avec les "interstitial chiasmata".  $\times \pm 2500$ . Fig. 9. *Sweetia perennis*. L'anaphase hétérotypique montrant les groupes à 12 chromosomes pendant leur ascension vers les pôles respectifs.  $\times \pm 2500$ .

1) Comparer: MOFFET, A. A., Cytologia, 1932, p. 30, fig. 6.  
2) BELLING, J., 1931.

*Sweetia*, au lieu de paires, aux chromosomes disposés en V, des paires de chromosomes attachés l'un à l'autre par leurs deux bouts—qui sont fréquemment unis par deux fins tractus (fig. 8b)—et formant des ellipses ou cercles (fig. 8a).

La définition du nombre de chromosomes chez *Sweetia* ne représente pas de telles difficultés comme chez les *Gentianes*, chez lesquelles on a pu définir approximativement<sup>1)</sup> seulement le nombre de chromosomes. Il est relativement facile de définir chez *Sweetia* aussi bien le nombre de gemini (stade de diacinèse) que le nombre de chromosomes univalents (anaphase méiotique, fig. 9) à condition que la cinèse se fasse normalement. Ce nombre est de 12 et se rapproche alors du nombre observé par OEHLER chez *Voyriella parviflora*<sup>2)</sup> et par SAKAI chez *Gentiana Makinoi*.<sup>3)</sup>

### Résumé

1. La paroi externe du sac pollinique de *Sweetia* diffère, au point de vue de la structure, de la paroi des *Gentianes*, étudiées jusqu'à présent (*G. asclepiadea*, *G. Fetisowi* et *G. lutea*), car elle est formée d'un plus grand nombre d'assises (6-7).

2. Tapetum est formé de deux couches, ce qui permet de distinguer une couche fondamentale, év. couche de "transfusion". Les cellules de ces deux couches restent uninucléaires.

3. Les chromosomes méiotiques chez *Sweetia perennis* présentent, malgré leur petitesse, une structure "chromomérique" depuis leptotène jusqu'au diplotène précoc.

4. Pendant la diacinèse les chromosomes homologues forment le plus souvent des couples parallèles en forme de V; souvent les couples s'unissent par deux bouts ce qui mène à la formation des ellipses ou des cercles; enfin un des couples prend habituellement la forme d'une tenaille fermée, avec "interstitial chiasmata".

5. Le nombre de geminis est de 12 et le nombre d'univalents dans l'anaphase (ou au stade des diades) est également de 12. Ce nombre s'approche du nombre de chromosomes établi par OEHLER pour *Voyriella parviflora* ou par SAKAI pour *Gentiana Makinoi*.

Institut de Botanique Générale de  
l'Univ. J. Piłsudski à Varsovie

1) TISCHLER, G., 1931, p. 170; 1935/36, p. 36.

2) OEHLER, E. (1927) en parle de 10-14.

3) En se basant sur le nombre somatique, obtenu par SAKAI (1934), TISCHLER (1935-36) fournit le nombre de 13.

## Littérature

1. Belling, J., 1928. Univ. Cal. Publ. Bot., V. 14.
2. — 1931. Univ. Cal. Publ. Bot., V. 16.
3. Darlington, C. D., 1935. Proc. Roy. Soc. London, V. 118.
4. Guérin, P., 1924. Comp. R. Ac. Sc., Paris, T. 179.
5. Heitz, E., 1935. Zeitschr. ind. Abst. u. Vererbl., Bd. 70. H. 3/4.
6. Hermans, C., 1936. La Cellule, T. 45. F. 1.
7. Moffet, A. A., 1932. Cytologia, V. 4, No. 1.
8. Ohler, E., 1927. Planta, Bd. 3.
9. Schaffstein, G., 1935. Zeitschr. f. Zellforsch. u. mikroskop. Anat., Bd. 22, H. 3.
10. Taylor, W. R., 1931. Amer. Journ. of Botany, V. 18.
11. Tischler, G., 1931, 1935/36. Tabulae Biologicae, Bd. 7, Bd. 12.
12. Wóycicki, Z., 1932, 1933, 1935. Acta Soc. Bot. Pol., V. 9, 10, 12.

## Cytogenetic Studies in Artificially Raised Interspecific Hybrids of *Papaver*. VI.

### The trigenomic hybrids of *Papaver*<sup>1)</sup>

By

K. Yasui

(With 2 Plates and 19 Text-figures)

#### Introduction

In the previous papers (1936 a and b) I have shown that 1) *Papaver orientale* has no homologous chromosomes in common with *P. somniferum*; 2) the plant No. 41-8 is a hybrid of *P. orientale* and *P. bracteata*; 3) the haploid chromosome set of *P. orientale* has three genomes,  $O_1O_2O_3$ , and  $O_1$  and  $O_2$  are at least partially homologous to each other, being capable of forming 7 gemini, but it was left undecided whether  $O_3$  is homologous to  $O_1$  and  $O_2$  or not; and 4) the genome  $O_3$  has 6 homologous chromosomes in common with the genome B.

*P. somniferum* was pollinated with the pollen of the plant No. 41-8 and many good seeds were obtained. Almost all of the  $F_1$  plant, however, died before the flower buds grew to a suitable stage for study, while some of them developed no flower buds, though they grew rather vigorously. Thus only two plants were secured for study. One of these two plants 41-124-3 showed several karyological peculiarities which suggest little affinity between the two parent plants.

#### Material and Method

The flower buds in a suitable stage of meiotic division were fixed with CARNOY's aceto-alcohol (1:3) mixture and transferred in 70% alcohol in which they were preserved. Also fresh material of PMC was stained with aceto-carmin with very good results, comparable with those of preserved materials. The material gradually swelled however as time elapsed, so as to make it difficult for us to speak of the natural size of the chromosomes.

To determine the number and other characters of somatic chromosomes the root-tips of seedlings were fixed with NAVASHIN's fixative. Microtome sections were stained with HEIDENHAIN's iron-alum haematoxylin.

1) Contributions from the Divisions of Plant-Morphology and of Genetics, Botanical Institute, Faculty of Science, Tokyo Imperial University, No. 180.

All drawings were made, using a camera lucida with a ZEISS objective, H.I.  $\times 100$ , N.A. 1.3, and ocular K. 12. They were reduced to 2/3 in reproduction. All photomicrographs were taken with a ZEISS objective, H.I.  $\times 100$ , N.A. 1.3 and LEITZ periplane ocular  $\times 10$ , and reproduced in the original size.

### Observation

1. *External characters.* The  $F_1$  plants (Text-fig. 1), being rather slender were not so vigorous as the parent plants. The stem was tomentous like the male parent, the leaves stiff; several floral shoots appeared and branched copiously, producing many buds which however mostly withered in a premature condition, so that only a few of them were able to bloom. The flower was smaller than those of either of the parents; the colour of the petal was orange-red with purple spots on the base, resembling that of the male parent. Among such flowers there were some in which the pistils and some

of anthers were abortive. The plants grew for several years, though they had shorter lives than the male parent, and died off without giving seeds.

#### 2. *Somatic cell.*

The nucleus of the somatic cell when stained showed deeply stained dots at the periphery of the nuclear cavity and near the surface of the nucleolus. In the early prophase the differentiation of the chromosomes starts from these deeply stained dots. The

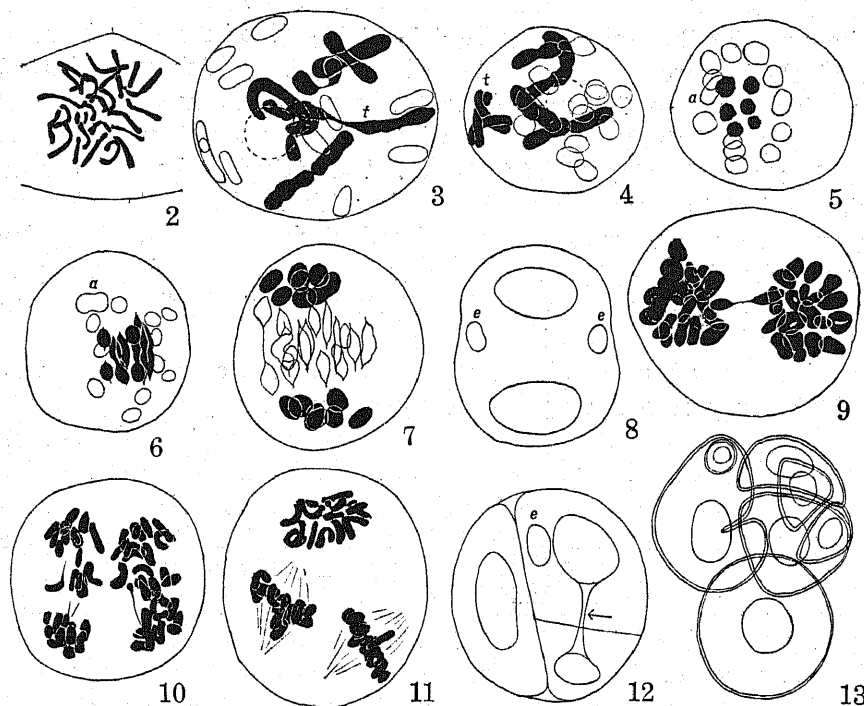


Text-fig. 1. The  $F_1$  plant, artificially raised hybrid, *P. somniferum*  $\times$  the plant No. 41-8.

chromosome number in the root-tip cells of several seedlings was found to be 25, that is the sum of the gametic chromosome

numbers of both parents (Text-fig. 2, and Plate 43, Fig. 1). Besides 26 chromosomes were counted in a mature plant from which the microsporocyte material of the present investigation was mostly obtained. The additional one chromosome might have been derived from the male parent, but not from an irregular division of the somatic cells in the  $F_1$  plant, though the irregular division occurred often in this plant. There is a possibility in PMCs of the male parent that there may occur non-disjunction of certain gemini or duplication of chromosomes.

3. *Cytomixis*. In the aceto-carmin-smear material, some PMCs



Text-figs. 2-13. 2. Polar view of the metaphase of a root-tip cell; 25 chromosomes seen. 3-13. PMCs of the  $F_1$  plant No. 41-124. 3, diplophase, 6 bivalents (black), one trivalent (t) (black), and 11 univalents (blank) seen, dotted circle shows the nucleolus, two bivalents attached to a nucleolus. 4, Diaphase, t shows a ring shaped trivalent. 5, 1st meiotic division, polar view, 6 bivalents at the center (black), other chromosomes at the peripheral region of the equator of the spindle. 6, 1st meiotic division, side view, 6 bivalents (black) at the equator, others scattering around them; one univalent is not shown. 7, 1st meiotic division, side view, separated bivalents and trivalent at the poles, univalents (blank) are dividing at the equator; two excess chromosomes present. 8, interphase, two small extra-nuclear chromosomes es shown at the equatorial region of the 1st spindle. 9, 2nd meiotic metaphase, polar view, a chromosome bridge shown between two daughter nuclei. 10, 2nd meiotic division, later stage. 11, 2nd meiotic division, 3 equatorial plates formed. 12, a triad, an arrow points to the chromosome bridge, e shows extra-small nucleus. 13, 7 pollen grains, produced in one PMC seen.



showed cytomixis (Plate 43, Fig. 2). It was caused certainly as an artifact by the pressure applied by the smear. But it is interesting as showing that the 'atractoplasm' (FUJII 1926) gets into another cell cavity together with the chromosomes, but does not mingle with the cytoplasm of the neighbouring cell. In the diaphase, intranuclear substance can easily be transferred into the neighbouring cell through pits in the wall.

4. *Pollen mother-cell*. In this  $F_1$  plant besides the ordinary PMCs there are peculiarly enormous ones. In addition to them, abortive-PMCs generally are found in the same anther.

5. *Meiosis in an ordinary PMC*. In the meiotic phases they show mostly the constitution  $6_{II} + 1_{III} + 11_I$  (Text-fig. 3). Four of 6 bivalents were larger than the remaining two which are spherical in shape in the 1st metaphase. The trivalent association was generally a chain (Text-fig. 3, *t*), but in a few case they associated in the form of a ring (Text-fig. 4, *t*). This trivalent has never been observed at the equator simultaneously with the bivalents. At that stage there was observed always a bivalent chromosome at the polar region (Text-figs. 5, 6). Sometimes a bivalent appeared at one pole and a univalent at the other as if that were derived by the separation of a trivalent (Plate 43, Fig. 5).

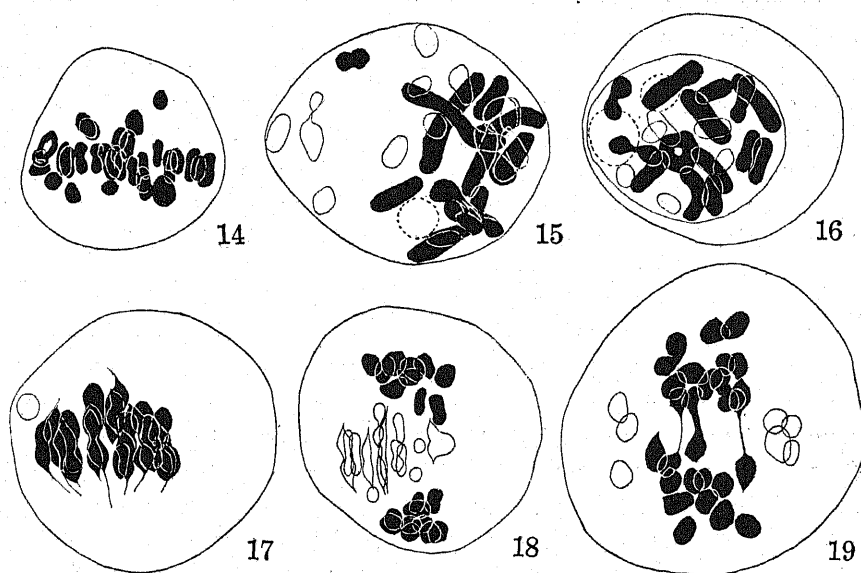
When the bivalents begin to separate at the anaphase the univalents come into the equator moving along the periphery of the nuclear cavity, come into the equator and arrange themselves in a peripheral circle. In such stage the polar view of the aceto-carmin material shows that the central bivalents are stained deeper than the peripheral univalents, as if the former had a stronger power of adhesion to the stain (Plate 43, Fig. 4), but in the side view, neither the bivalents nor univalents show any such difference. The difference in the staining colour in the polar view is probably due to the difference of the thickness of the chromosomes.

In the metaphase of the univalents, some of them are situated nearer to one pole than to other. They may pass into one pole without separation. In other cases, the divided halves may separate and the one half precede while the other retards, as they are differently distant from the two poles (Plate 43, Fig. 7, *a* and *b*). Thus almost always some chromosomes retard, resulting in the appearance of extra-nuclear chromosomes (Text-fig. 8), or a chromosome bridge, which often joins two daughter nuclei even at the stage in which the latter are in the 2nd division (Plate 43, Figs. 8 and 9; Text-fig. 9). Restitution nuclei were also observed.

In some PMCs a more or less complete cell wall formation occurred after the 1st meiotic division (Plate 43, Fig. 14), which is

unusual in the PMC of *Papaver*. The 2nd meiotic division occurred in such divided daughter cells just as in the case where such cell-wall formation is omitted. Consequently there were observed both simultaneous and successive tetrad-formations in one and the same anther, as I already described in an  $F_1$  plant between *P. somniferum* and *P. orientale* (YASUI 1931).

In the 2nd meiotic division almost all chromosomes, those derived from bivalents as well as those from univalents, entered in the formation of the equatorial plate simultaneously (Plate 43, Figs. 9 and 13; Text-fig. 9). While the divided halves of the bivalents separate first and go to both the poles, undivided ones (divided halves of the univalents in the 1st division) are left at the equatorial region. When the former get into a later anaphase, the latter follow them toward both poles with a random distribution (Plate 43, Fig. 10; Text-fig. 10). Sometimes three equatorial plates instead of two are formed in a PMC which consists of about the same number of chromosomes (Text-fig. 11). Thus the second division is also irregular, so that almost all PMCs show more or less irregular tetrad formations, extra-nuclear chromosomes, lagging



Text-figs. 14-19. PMCs of the  $F_1$  plant No. 41-124. 14, probably a restitution nucleus, side view of 2nd meiotic division. 15, one enormous nucleus, containing a few tetravalents. 16, an enormous PMC in which 14 bivalents (black), 6 univalents (blank) and 2 nucleoli (dotted circle) are seen. 17, another large PMC, many bivalents in metaphase and one univalent (blank) shown, one or 2 remaining univalents not being shown. 18, 1st meiotic division of large PMC, side view. 19, another large PMC, many bivalents (black) seen already separated, while three are in division, and univalents (blank) are about to enter into the equator.

chromosomes, chromosome bridges, dyads, triads, pentads, hexads; miniature grains mixed with large grains, etc. being found mixed with regular tetrads.

There were observed in some PMCs about 30 univalent-like chromosomes, of which most were arranged at the equator while the rest were scattered around them (Text-fig. 15). Such a PMC is probably the restitution cell of the 1st meiotic division. From the number and the structure of the chromosomes we can conclude that the dividing chromosomes at the equator are the separated halves of bivalents and not divided univalents in the 1st meiotic division, while here non-dividing and scattering ones are the divided halves of the univalents in the 1st division. Such PMCs will give rise to dyads, having two different chromosome constitutions in their nuclei, because the distributions of the divided univalents into two daughter nuclei are different.

6. *Enormous PMC.* a) Some of large PMCs had nuclei twice as large as those of the ordinary PMC. Some of them had a notch on the cell wall which suggests the imperfect division of an archesporial cell (Plate 44, Fig. 15). The size of the cell and nucleus, the chromosome number, their configuration, and the notch show that this PMC has a restitution nucleus formed by the irregular division of the premeiotic division of an archesporial nucleus. b) A large PMC accompanied by a small abortive one was found (Plate 44, Fig. 16). They are sister cells produced by unequal nuclear and cell division of an archesporial cell. In the metaphase of the 1st meiotic division of this large cell, the chromosomes are found mostly as bivalents, but few of them as univalents (Text-fig. 17). The division of such a nucleus is more regular than that of the ordinary ones, and passes into an interkinesis rather normally (Plate 44, Fig. 17). The small sister cell had a completely resting nucleus whose size corresponded to one or two chromosomes. Such small cells accompanying larger sister cells were found several times in several anthers (Plate 44, Fig. 18, c, and Fig. 19). Some of the small cells were fairly large in size, but the nucleus did not show any trace of meiosis (Plate 43, Fig. 12), while some small sister cells were in the meiotic prophase (Plate 44, Fig. 20). c) There was found another large PMC in which two unequal nuclei were dividing (Plate 44, Fig. 21). The division in the small nucleus is rather regular, while that in the large nucleus is quite irregular. It is difficult to determine whether this PMC shows the 1st meiotic division or the 2nd division. If the former is the case, then the PMC was derived from an archesporial cell in which an irregular premeiotic nuclear division occurred without cytokinesis. But if the latter is the case, then the

PMC was an enormous one having a large restitution nucleus which divided into two unequal daughter nuclei.

These irregularities were found in several anthers and also in the several flowers gathered on different days. Though the chromosome numbers in such large PMCs were not the same, the number of the bivalents was more than seven, while the number of the univalents was generally smaller than that of the ordinary PMC (Plate 44, Figs. 16, and 22; Text-figs. 15-17). These facts show that the large nuclei contained a larger number of the homologous pairs of chromosomes than those of the ordinary PMC.

The stages of the meiotic division of the PMCs in one and the same anther were not the same generally, and the division into the enormous PMC was generally in an earlier stage. The 2nd division in the enormous PMC was not observed.

Thus there were many kinds of PMCs in one and the same anther, and their division was also irregular and moreover the mode of cytokinesis was not the same, consequently the tetrad formation is quite complicate, dyads, triads, pentads, extra-nuclear chromosomes, miniature pollen grains, chromosome bridges, etc. being found. The chromosome numbers in these pollen grains are different from each other, and the combinations of the chromosomes of different genomic derivation can be of extreme complexity.

### Discussion

1. *The genom constitution.* Plant 41-8 is a tetraploid, whose genom constitution was analysed as  $O_1O_2O_3B$ , where  $O_1$  and  $O_2$  are homologous to each other (YASUI 1936b). Thus we may say that there are genomically two different pollen grains,  $O_1O_3$  and  $O_1B$ , while  $O_3$  and  $B$  have 6 homologous chromosomes. If *P. somniferum* had only one genom in its gametic chromosome sets which form gemini with the chromosomes of *P. orientale* in  $F_1$  plant (YASUI 1922, LYJUNGDAHL 1923, SANSOME and PHILP 1930), and if seven of 11 chromosomes in *somniferum* are homologous to  $O_1$ , then the remaining 4 must have their partners to form gemini in  $O_3$ , because, if it is not so, they must have partners in  $O_2$ , while  $O_2$  is homologous with  $O_1$ ; consequently the 4 chromosomes in question must have their homologues in the other 7 chromosomes of *somniferum*. This consideration is however not in accord with the above mentioned assumption that *P. somniferum* had only one genom. If we accept the assumption, then we must have at least 10 or 11 gemini in the  $F_1$  plant (*P. somniferum*  $\times$  41-8), but as a matter of fact we have only 6 bivalents and one trivalent.

One might consider that the 6 bivalents and the one trivalent have been derived from the male parent and the 11 univalents from the female parent. Then either  $O_1$  and  $O_3$  or  $O_1$  and B must be homologous. Thus it becomes highly probable that *P. orientale* is an autohexaploid. But such a view can not explain the fact that the  $F_1$  plant of *P. somniferum*  $\times$  *P. orientale* has 11 bivalents and 10 univalents. According to this consideration the  $F_1$  plant of *somniferum* and *orientale* must have its chromosome constitution  $7_{II} + 18_I$  or  $7_{III} + 11_I$ . Therefore this view is also inadmissible.

According to my view (YASUI 1936b) *P. Somniferum* has no homologous chromosomes in *P. orientale* and *P. somniferum* has 4 pairs of chromosomes in its own chromosome set which form gemini in  $F_1$  plant of *P. somniferum*  $\times$  *P. orientale* and also in the  $F_1$  plant *P. somniferum*  $\times$  *P. lateritium*; therefore 4 of the 6 bivalents in the  $F_1$  plant (*P. somniferum*  $\times$  41-8) must have derived from the female parent and the 2 bivalents and the one trivalent from the male parent.

From the presence of two bivalents and one trivalent, we may conclude that there are three more or less homologous pairs in the genomes  $O_1$  and  $O_3$  and in  $O_1$  and B.

2. *Irregular premeiotic division.* KARPECHENKO (1927) described a binucleate PMC in *Raphanus-Brassica* hybrid. He also mentioned the possibility of the production of PMC with a double number of chromosomes, by a suppression of the nuclear division after the longitudinal splitting of chromosomes had already occurred. FUKUSHIMA (1931) described the meiosis of diploid, tetraploid, and octoploid PMCs in one and the same anther in a race (Japanese name Miduna) of *Brassica japonica*, though he did not ascertain the origin of these duplications. However, he has concluded with MORINAGA who has found a similar case in Kaya-kabu (a race of *Brassica Rapa*). (unpublished) that the duplication might have occurred in some archesporial cells. SHIMOTOMAI (1931, and 1933) also described the enormous PMC in the species hybrids of *Chrysanthemum*, considering the possibility of their formation by irregular division of archesporial cells. The case of *Papaver* now under discussion gave an actual proof of the irregular division of the archesporial cells. What would be the cause of such an irregular division?

Since NĚMEC (1910) has reported on the artificially induced irregular behaviours of mitosis, there have been so many similar observations made under various artificial conditions (temperature, narcoses, X-radiation, radium-rays etc.), as to be too numerous to mention here. In the present case of *Papaver* there has been no such unusual external conditions found, so that the irregular divisions are

to be ascribed to an internal condition or at least to strong susceptibility to a change in the external influences.

The genom constitution of the male parent (41-8) is  $O_1O_2O_3B + 1$ , in which chromosomes in  $O_1$  and  $O_2$  can form gemini, and 6 chromosomes in  $O_3$  can associate with 6 in B. In the meiosis in the Plant 41-8, they have random assortments: therefore if these associations are due only to their partial homology, the gene constitution of the pollen grains must be very complicated, and may cause the abortion of the pollen grains. But actually the pollen grains are rather healthy, therefore such a random assortment does not affect the viability of the pollen grains, but they might exert influence on some characters in the sporophyte. The complex constitution of chromosome sets in a nucleus, especially the difference in the rapidity of the separation movement of the chromosomes may produce the irregularities even in the premeiotic division. In an extreme case they may give a chance for the separation of one genom from the other.

Still further there is another possibility that the premeiotic irregular division may be due to the effect of the aneuploid constitution of the plant. SHIMOTOMAI's *Chrysanthemum* above referred to is also an aneuploid ( $7n-1$ ), while the case of FUKUSHIMA's *Brassica* is a true diploid. A further study of the 25 chromosome  $F_1$  plant is expected to throw some light on this point.

The pollen grains having diploid or some aneupolyploid\* chromosome sets may be viable, and then they may give a change for the origin of a polyploid or aneupolyploid sporophyte.

3. *Tetrad formation.* I have described before (1931) an example of *Papaver* in which successive and simultaneous tetrad divisions occurred in one and the same anther. It was considered as caused by the influence of unusually cold weather. Such simultaneous occurrence of the two kinds of tetrad divisions in an anther happened in the present material too. But in the present case it happened in several buds and the special external causes are not known. At any rate, it is a fact that in case of longer interkinesis and slowness of division the successive division takes place.

As I have mentioned in my previous paper (YASUI 1931) these types of the tetrad formation are not specifically fixed characters of certain plant groups, but they may vary under certain external or internal conditions.

\*I should like to propose this term to denote a chromosome set having plus or minus chromosome in addition to certain euploid chromosomes; the word 'poly' may be substituted by a term denoting a number, namely di- tri- etc.; also the word 'hypo' or 'hyper' may be prefixed (e.g. 'hypoaneudiploid', 'hyperaneutriploid', etc.).



4. *The abortive-PMC.* The abortive-PMC is a daughter cell produced by a premeiotic irregular division of an archesporial cell. It is a question why its nucleus does not show any sign of division while the sister nucleus is in the meiosis. Several cases of the artificial suppression of the processes of mitosis and meiosis have been described by various authors. But in the present case such abnormal external influences cannot be considered, because the sister cells and other ordinary PMCs were showing the normal meiotic division, so that the PMC-abortion must be ascribed to some internal cause or causes. The smaller number of the chromosomes in the nucleus alone cannot be the cause, because some small sister cells were in prophase. In the meiotic phases sometimes a few chromosomes, even one, can form a nucleus which can show 2nd division, and some retarding chromosomes do not enter into 2nd division and generally degenerate in the cytoplasm. It is unknown yet how the two cases are different in the nuclear conditions. The only cause we can now consider is that in the latter case the chromosomes are naked or not enclosed in the 'atractoplasm'. The same cause may be present in some of the abortive-PMCs, but it is not applicable in all cases, because some of the latter are large enough and enclosed within 'atractoplasm'.

Finally we may consider certain chromosomes containing genes which are concerned with the process of meiosis.

### Summary

1. A hybrid between *P. somniferum* and the plant No. 41-8 (a supposed natural hybrid between *P. orientale* and *P. bracteata*) was produced. The  $F_1$  plant resembled the male plant mostly in its external characters, but a few characters resembled those of the female parent.

2. The chromosome number in the root-tip cells in many young  $F_1$  seedlings was 25 which is the sum of the gametic numbers of the parent plants, but the chromosome number of a matured  $F_1$  plant, from which the material of this cytological study was obtained, was 26. The one additional chromosome may have been derived from the male parent.

3. There were found mainly two kinds of PMCs, an ordinary and an enormous one, in the  $F_1$  plant. The chromosome constitution in the ordinary PMC was  $6_{II} + 1_{III} + 11_I$ . The meiosis in these PMCs was in general of the *Triticum* type and produced dyads, triads, pentads, hexads together with tetrads, also showing other irregularities, such as extranuclear chromosomes, chromosome bridge, lagging chromosome, miniature pollen grains, etc.



The relationships of the genomes in the  $F_1$  plant were discussed.

Enormous PMCs derived from the archesporial cells by irregular premeiotic divisions were observed. Chromosome numbers in them were various, showing more bivalents and less univalents than those of the ordinary PMC. As a result some multiploid or aneuploid gametophyte may be produced.

The abortive-PMC is the small sister cell of the enormous one. It has always the resting nucleus showing no trace of the meiosis. This suppression of meiotic division is considered as due to a gene or genes which govern the meiotic process.

Here the writer wishes to express her best thanks to Prof. K. FUJII, by whose suggestion these studies were started, for his valuable advice throughout the course of the work. The expence of carrying out this study was partly defrayed out of a grant from the Japan Society for the Promotion of Scientific Research, to which the writer's thanks are due.

Botanical Institute, Faculty of  
Science, Tokyo Imp. University  
and Botanical Department, Tokyo  
Higher Normal School for Women

#### Literature

- Fujii, K. 1931. Saibogaku no Kwako oyobi Genzyô. (Japanese) (Cytology in the past and present). Iwanami Tokyo.
- Fukushima, E. 1931. Formation of diploid and tetraploid gametes in *Brassica*. Jap. Jour. Bot. 5: 273-283.
- Karpechenko, G. D. 1927. The production of polyploid gametes in hybrids. Hereditas, 9: 349-368.
- Ljungdahl, H. 1922. Zur Zytologie der Gattung *Papaver*. Svensk Tidsk. 16: 103-114.
- Némec, B. 1910. Das Problem der Befruchtungsvorgänge und andere zytologische Fragen.
- Sansome, F. W. and Philp, J. 1932. Recent advances in plant genetics. London.
- Shimotomai, N. 1931. Über die abnorme Reduktionsteilung in P.M.Z., die einen riesigen Kern oder überzahlige Zwergkerne enthalten (Japanisch). Bot. Mag. (Tokyo), 43: 356-363.
- 1933. Zur Karyogenetik der Gattung *Chrysanthemum*. Jour. Sci. Hiroshima Univ. Ser. B. Div. 2, Vol. 2, Art. 1.
- Yasui, K. 1921. On the behaviour of chromosomes in the meiotic phase of some artificially raised *Papaver* hybrids. Bot. Mag. (Tokyo) 35: 154-167.
- 1927. Further studies on genetics and cytology of artificially raised interspecific hybrids of *Papaver*. Bot. Mag. (Tokyo) 41: 235-261.
- 1931. Cytological studies in artificially raised interspecific hybrids of *Papaver*. III. Unusual cases of cytokinesis in pollen mother-cells in an  $F_1$  plant. Cytologia 2: 402-419.
- 1936a. Cytological Studies in artificially raised interspecific hybrids of *Papaver*. IV. On the genom of *Papaver* especially of *P. somniferum* and *P. orientale*. (Japanese). Proc. 4th Congress of Bot. Soc. Japan. Bot. Mag. (Tokyo) 50: (13).
- 1936b. Cytogenetic studies in artificially raised interspecific hybrids of *Papaver*. IV. Interspecific hybrids of *P. orientale* L. and *P. bracteata* LINDL.

## Explanation of Plates

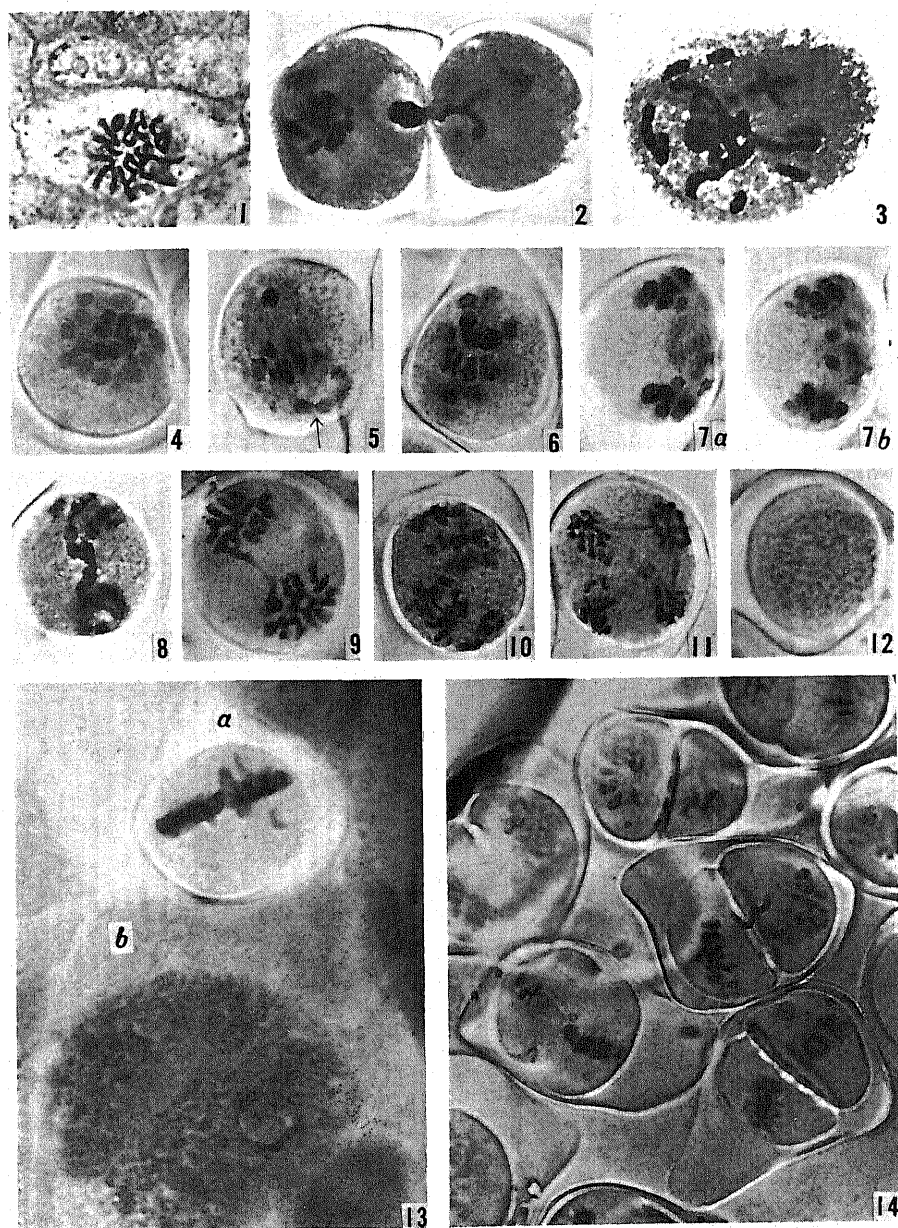
All figures are photomicrographs taken with ZEISS objective, H.I.  $\times 100$ , N.A. 1.3 and LEITZ periplane ocular  $\times 10$ .  $\times 1000$ .

## Plate 43

- Fig. 1. A polar view of a metaphase and a resting nucleus from a seedling of the  $F_1$  plant No. 41-124 (*S. somniferum*  $\times$  Plant No. 41-8).  
 Figs. 2-12. PMCs of the  $F_1$  plant No. 41-124.  
 Fig. 2. 2 PMCs, showing cytotoxicity.  
 Fig. 3. A diaphase.  
 Fig. 4. Polar view of a 1st meiotic division, bivalents in the center and the univalent at the periphery of the equatorial region.  
 Fig. 5. 1st meiotic division, side view, showing bivalents at the equator and some univalents scattered around them; a bivalent at one pole, and a univalent at the other. An arrow points out a bivalent at the pole.  
 Fig. 6. 1st meiotic division, equatorial side view, four large bivalents are about to separate their partners.  
 Fig. 7, *a* and *b*. Side view of the 1st meiotic division at two optical planes; separated bivalents nearly reached the poles, while the remaining ones at the equatorial region.  
 Fig. 8. Interphase, a chromosome bridge is seen.  
 Fig. 9. Metaphase of the 2nd meiotic division, polar view; chromosome bridge between the two daughter nuclei seen.  
 Fig. 10. A later stage, separated halves of the bivalents at the poles, the halves of univalents seen at equator.  
 Fig. 11. A still later stage, showing chromosome bridges between two daughter chromosome groups of the 2nd division and also those produced by the first division between the daughter nuclei of the first division.  
 Fig. 12. A PMC having the completely resting nucleus.  
 Fig. 13. A large tapetum cell (*b*) having two enormous nuclei situated very close to each other, and a PMC in 2nd meiotic metaphase in side view (*a*).  
 Fig. 14. A group of PMCs from one and the same anther showing their nuclei in the 2nd meiotic metaphase. The three cells to the right show that they divided completely after the 1st meiotic division, while the two to the left show incomplete cytokinesis after the 1st division.

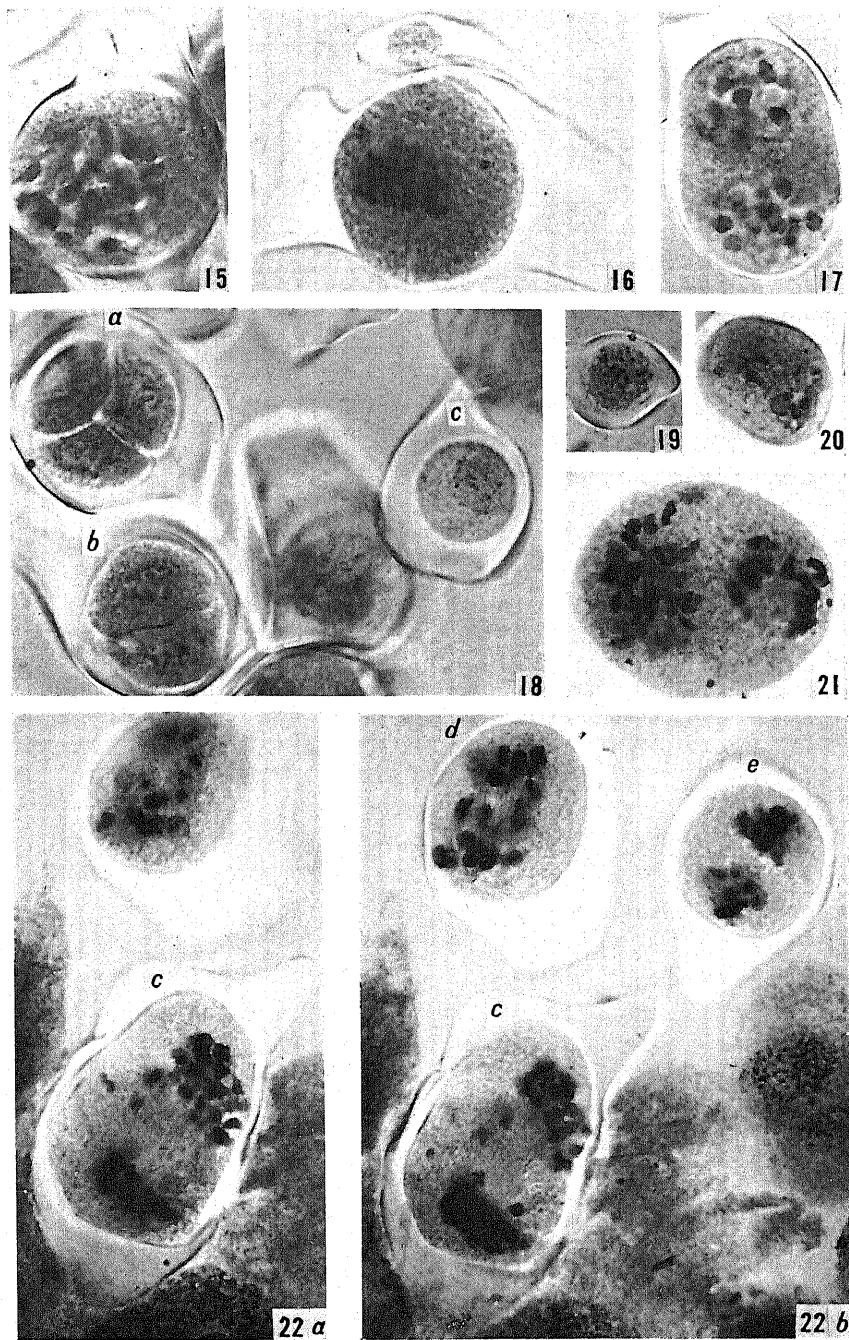
## Plate 44

- Fig. 15. A large PMC having one large nucleus in diaphase, the wall at the upper side of the figure shows the trace of an incomplete cell division.  
 Fig. 16. A large PMC accompanying a miniature sister cell (abortive-PMC); the larger cell in the 1st meiotic metaphase shows many bivalents and a few univalents.  
 Fig. 17. Interkinesis of a large PMC.  
 Fig. 18. PMCs from one and the same anther; *a* tetrad, *b*, dyad, *c*, abortive-PMCs having resting nuclei.  
 Fig. 19. A very small abortive-PMC, having a resting nucleus.  
 Fig. 20. A small PMC having chromosomes less than in an ordinary PMC.  
 Fig. 21. A large PMC, having two dividing unequal sized nuclei.  
 Fig. 22 *a* and *b*. 3 PMCs from one and the same anther in different optical levels. *c*, a large PMC in the telophase, a number of separated univalent-halves near the poles, and one univalent at the equator where the MLI (middle lamella initial) is seen; *d*, a PMC in the 1st meiotic phase; *e*, an ordinary PMC in the 2nd meiotic division.



Yasui: Cytogenetic Studies in Artificially Raised Interspecific Hybrids of *Papaver*. VI. The trigenomic hybrids of *Papaver*





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## Über das sogenannte Schrittwachstum der Zelle

Von

**W. A. Becker**

Aus dem Institut für Allg. Botanik Josef-Piłsudski-Universität Warszawa  
(Vorstand: Prof. Z. Wóycicki)

### I. Einleitung

Als ich nach meinen Untersuchungen über die Phragmoplasten-cytokinese (BECKER, 1932, 1934) auch andere Arten der Cytokinese und Protoplasmaumhütung untersuchte (*Chlamydomonas*, *Saprolegnia*; 1934), wurde ich auf manche Vorgänge aufmerksam, die eine nähere Untersuchung erforderten. Als Objekt für die Untersuchungen über Plasmaumhütung wählte ich *Basidiobolus ranarum*, bei welchem—wie aus der Literatur hervorgeht—vier verschiedene Formen von Membranbildung de novo beobachtet wurden:

1. Auf der Oberfläche des sich kontrahierenden Protoplasten im Zusammenhang mit dem Schrittwachstum, wobei die Membran leere Kammern abgliedert (RACIBORSKI, KÜSTER, u.a.).
2. Im Innern des Plasmas der vegetativen Fäden im Zusammenhang mit der Kernteilung (OLIVE, RACIBORSKI u.a.).
3. Zellplatten-Cytokinese im Zusammenhang mit der Bildung von Kopulationsorganen (FAIRCHILD).
4. Umhütung der Palmella-Formen (RACIBORSKI, LEVISOHN u.a.).

*Basidiobolus ranarum* ist überdies ein klassisches Beispiel für sehr rasches und intensives Oberflächenwachstum der Membran, wobei das Wachstum angeblich ohne Anteil des Turgors erfolgt. Alle diese Umstände liessen erwarten daß *Basidiobolus* für die Untersuchung der uns interessierenden Vorgänge sich besonders eignen wird. Dieser Pilz zeichnet sich bekanntlich durch seine großen Zellen und Kerne aus, läßt sich leicht in vivo beobachten und eignet sich für Vitaluntersuchungen (RACIBORSKI, WÓYCICKI, ULEHLA, MORAVEK, BECKER, SKUPIŃSKI u.a.).

Das Material mit welchem wir arbeiteten wurde in Reinkultur aus dem Mykologischen Institut in Baarn bezogen. Die Kulturen wurden entweder in ERLÉNMEYER-Kolben und PETRI-Schalen (Makrokulturen) oder auf Deckgläschen in Feuchtkammern (Mikrokulturen) angelegt. Nährlösungen wendeten wir verschiedene an: flüssige, feste gefärbte, und farblose. Im Bedarfsfalle nahm ich zu verschiedenen experimentellen Hilfsmethoden, wie Vitalfärbung, Plasmolyse,



Mikrurgie u.s.w. Zuflucht. Die vorliegende Publikation enthält Beobachtungen über das sogenannte Schrittwachstum der Zelle.

## II. Über das Schrittwachstum der *Basidiobolus*-Zelle

Die bereits seit HOFMEISTER (1867, S. 45) bekannte Tatsache, daß an der Spitze röhrenförmiger Zellen mit unbegrenztem Wachstum (Algen, Pilze, Pollenschläuche) Protoplasma sich ansammelt, wurde durch RACIBORSKI (1907) an *Basidiobolus*, wo überdies die Terminalzelle durch eine Querwand rhythmisch abgegliedert wird, genauer untersucht und als Schrittwachstum bezeichnet. Der Mechanismus dieses Wachstums und der Plasmakontraktion wird von allen Autoren auf dieselbe Art und Weise gedeutet.

HOFMEISTER (S. 45; vgl. auch KÜSTER, 1929, S. 24) schreibt darüber: Eine langsame Wanderung des Protoplasmas, auch desjenigen des relativ ruhenden Wandbelegs kommt allen Algen und Pilzen zu, deren Vegetationsorgane röhrenförmige Zellen mit unbegrenztem Wachstum der Spitzen sind: den Siphoneen, Saprolegnieen, und Verwandten; und in allen irgend größere Länge erlangenden Pollenschläuchen. Die älteren hinteren Teile derselben werden endlich vom Protoplasma völlig entleert. Nachdem die innere Masse des Protoplasmas schon früher nach der wachsenden Spitze der fadenförmigen Zellen hin sich begab, zieht endlich auch der Wandbeleg von der Innenfläche der Zellhaut sich zurück, sein Volumen verkleinert, und rückt nach derselben Richtung hin weiter. Ältere Teile der Fäden von *Vaucheria*, *Saprolegnia*, *Pilobolus* werden so allmählich protoplasmaleer“.

Aus den zit. Beobachtungen HOFMEISTER's folgt, daß das ganze Plasma der röhrenförmigen Zelle, den Wandbeleg inbegriffen, an der Spitze sich sammelt, so daß die älteren Teile der Zelle leer bleiben. Einen ganz analogen Wachstumsmechanismus stellte STRASBURGER (1882) bei Pollenschläuchen fest. Bei *Basidiobolus* wird überdies das an der Spitze angesammelte Plasma durch eine Querwand abgetrennt. Der plasmaleere Teil des Fadens scheint dadurch in eine Reihe leerer Kammern septiert. Nach der Schilderung von RACIBORSKI findet dieser Vorgang folgenderweise statt:

„Unter Wasseraufnahme und Vergrößerung der Vacuolen wird die Zelle bei bleibender Dicke immer länger, um nachträglich durch Kontraktion des Protoplasmas, Verlassen durch dasselbe des proximalen Endes der Zelle, unter Flüssigkeitsaustritt aus der Zelle und entsprechender Verkleinerung des Vacuolenraumes kürzer zu werden. Nach der Kontraktion wird das kontrahierte Plasma durch eine neugebildete Querwand von dem leer bleibenden basalen Raum der Zelle abgetrennt“ (1907, S. 902).

Der Plasmahaut entledigt kommt die Vacuole aus dem leeren basalen Teil der Zelle notwendigerweise in Berührung mit der Außenwelt und gibt ihren Inhalt ab.

„Wird die basale Vacuole der wachsenden *Basidiobolus*zelle sehr groß, ihr wandständiger Plasmabeleg sehr dünn, so zieht sich derselbe zu einem oder zu einigen sehr dünnen und langen Strängen zusammen, welche durch die Hauptmasse des Protoplasmas eingezogen werden. Schon vor der Systole sind diese Stränge als dichtere Rippen auf dem ganz dünnen Plasmabeleg sichtbar, und zu

diesen Rippen zieht sich der dünne Plasmabeleg während der Systole zurück, wobei die Zellflüssigkeit in offene Kommunikation mit der Umgebung tritt“ (RACIBORSKI, 1907, S. 920).

Wie aus dieser Darstellung folgt, verläßt auch bei *Basidiobolus* ähnlich wie in den von HOFMEISTER angeführten Fällen das ganze Plasma den Wandbeleg inbegriffen, den Basalteil der Zelle. Das Schrittwachstum von *Basidiobolus* beruht somit auf einer rhythmischen Diastole und Systole des Plasmas, welches sich von den leeren Kammern, die es früher einnahm, durch Querwände abgrenzt.

KÜSTER betont (1935, S. 565), daß das Schrittwachstum der Zelle im Pflanzenreich viel mehr verbreitet, wenn auch noch unvollkommen erforscht ist. In diesem Sinne dürfen nach KÜSTER's Ansicht die Angaben PRINGSHEIMS (1873) und PFITZERS (1872) über das Wachstum bei *Achlya*, die Angaben KNIPE's (1928) über *Phyllodium*, WIEBEN's über *Taphrina* und LAIBACH's über *Ustilago* gedeutet werden. Was den Mechanismus des Schrittwachstums anbelangt, vertritt KÜSTER dieselbe Meinung wie HOFMEISTER und RACIBORSKI u.zw., daß das ganze Plasma sich an der Spitze der Zelle sammelt, während der Rest des Fadens leer bleibt. Über die Untersuchungen WIEBEN's schreibt KÜSTER: „Wenn der Schlauch mehrere „Zellen“ abgegliedert hat, so enthält er immer noch nur ein Kernpaar“...., wobei er das Wort „Zellen“ mit Anführungszeichen versieht. Weiters bemerkt KÜSTER „die anderen Kammern sind leer und die sie trennenden Wände sind offenbar dadurch entstanden, daß das Protoplasma sich in akropetaler Richtung zusammengezogen und die älteren Strecken der Hyphe leer zurückgelassen hat“. Auch LAIBACH nimmt in dieser Angelegenheit eine deutliche Stellung ein: „Stets befindet sich der gesamte Plasmahalt beider Sporidien an der Spitze des Schlauches und kammert sich immer wieder gegen den leeren Teil ab, so daß dieser deutlich und ziemlich regelmäßig septiert erscheint“ (S. 343).

Diese Auffassung über den Mechanismus des Schrittwachstums beeinflusste in starkem Grade die Entwicklung unserer Anschauungen über den Mechanismus des Membranwachstums während des Zellenwachstums. Da während der Diastole und der Systole sowie während der Vacuolenbildung und der Wasserausscheidung der Faden fort-dauernd in die Länge wächst (RACIBORSKI), mußte notwendigerweise angenommen werden, daß der osmotische Druck, entgegen gewissen Behauptungen, für die Membranwachstum keinesfalls unbedingt notwendig ist. Das Schrittwachstum der Zellen von *Basidiobolus* gilt daher in der botanischen Literatur als klassisches Beispiel für Membranwachstum ohne Anteilnahme des osmotischen Turgors (KÜSTER, 1935, S. 446).

Während unserer Untersuchungen über *Basidiobolus* sind wir indessen auf Erscheinungen gestossen, die uns zu einer anderen Stellungnahme in dieser Frage veranlaßten. Auf Grund einer Reihe von Beobachtungen konnten wir uns überzeugen, daß in den abgegrenzten Kammern immer ein deutlicher, manchmal recht dicker Wandbeleg zurückbleibt, daß wir somit in diesem Falle, entgegen den bisherigen Anschauungen, mit einer gewöhnlichen Abtrennung plasmaarmer, kernloser Zellen zu tun haben. Nachfolgend führen wir einige experimentelle Beweise für unsere Ansicht an.

#### a. Plasmolyse

Als Plasmolysierungsmittel wendeten wir 30% Glukoselösung bzw. hypertonische Saccharose- und  $\text{KNO}_3$ -Lösungen an. In allen Zellen erfolgt eine typische Plasmolyse, die meistens die Form der WEBER'schen Konkavplasmolyse annimmt. Diese Form der Plasmolyse ist für die „leeren“ Zellen charakteristisch. Schon das Plasmolysierungsverfahren allein genügt um die Anwesenheit einer Plasmahaut in den Zellen, die bisher als leere Kammern galten, nachzuweisen. Selbst in sehr langen Hyphen läßt sich das Plasma mittels Plasmolyse in allen Zellen nachweisen. Es ist nicht nur in demjenigen Teil des Fadens, der der Terminalzelle am nächsten liegt, sondern auch in den weiter liegenden Zellen vorhanden. Während der Plasmolyse erfahren die Fäden oft eine Drehung, die aber keinesfalls eine Folge der Drehung der Terminalzelle ist, da von zwei übereinander liegenden „leeren“ Zellen die proximale unverändert bleiben kann, während die entferntere bereits eine Drehung erfährt.

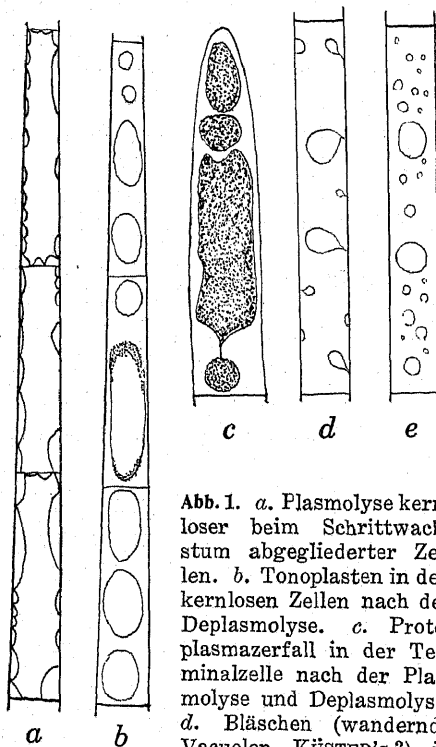


Abb. 1. a. Plasmolyse kernloser beim Schrittwachstum abgegliederter Zellen. b. Tonoplasten in den kernlosen Zellen nach der Deplasmolyse. c. Protoplasmazerfall in der Terminalzelle nach der Plasmolyse und Deplasmolyse. d. Bläschen (wandernde Vacuolen KÜSTER's?) in kernlosen Zellen alter Kulturen. e. Dieselbe Zelle (d) nach der Deplasmolyse.

Die plasmaarmen Zellen verbleiben in dem auf Abb. 1a dargestellten Zustande nicht lange. Sehr rasch erfolgt die weitere Abschnürung

des von der Wand losgelösten Protoplasmas und der Zerfall des ganzen Plasmaschlauches auf einige Tonoplasten, die im Bereiche der Zellmembran zu liegen kommen (Abb. 1b). Der Zerfall erfolgt in der Regel nach jeder Deplasmolyse. Nach jedem Plasmolysierungsverfahren treten in allen plasmaarmen Zellen mehrere oder weniger, größere und kleinere Tonoplasten auf. Auch in den Terminalzellen zerfällt der Protoplast in einzelne Stücke (Abb. 1c). Auf manchen Tonoplasten tritt an den Enden eine dickere Plasmaschicht auf (Abb. 1b).

In älteren, mehrere Monate alten Kulturen läßt sich mittels Plasmolyse nicht immer ein typischer Plasmaschlauch, wie auf Fig. 1a nachweisen. Dagegen tritt hier eine andere interessante Erscheinung auf. Unter der Einwirkung der Plasmolyse kommen auf der Innenseite der Membran kleine Bläschen zum Vorschein, die sich bewegen, wachsen und fortwährend ihre Form ändern (Abb. 1d). Sie erinnern sehr stark an die „wandernden Vacuolen“ von KÜSTER (1932, vgl. auch 1935). Die Bläschen lösen sich von der Membran los; nach stärkerer Plasmolyse und Deplasmolyse erhalten wir in der Zelle einen ganzen Schwarm derartiger winziger, in BROWNScher Bewegung begriffener, Bläschen (Abb. 1e).

### b. Vitalfärbung

Vitalfärbung mit „basischen“ Azinfarbstoffen ergibt kein nennenswertes Resultat, da die intensiv wachsenden Zellen in der Regel eine Entfärbung erfahren (BECKER u. SKUPIŃSKI). Während aber in den Terminalzellen unter Beihilfe eines traumatischen Reizes die Vacuolen sich umkehrbar färben lassen, gelingt dieser Versuch in den plasmaarmen Zellen fast niemals. Neutralrot und ähnliche Farbstoffe färben hier momentan eine Reihe winziger, im Wandbeleg bzw. in den Plasmafäden liegender Körnchen (Abb. 2a). Überdies färbt sich hier vor allem die Zellmembran.

Die geschilderten Ergebnisse stehen in keinem Widerspruch mit unserer These von der Gegenwart einer Plasmahaut unter der Zellmembran, da der Umstand, daß der Zellsaft mit Vacuolarfarbstoffen sich nicht färbt, keinesfalls als Beweis des Fehlens einer typischen Vacuole angesehen werden kann. STRUGGER (1935) wies nach, daß Neutralrot bei saurer Reaktion eben die Zellmembran

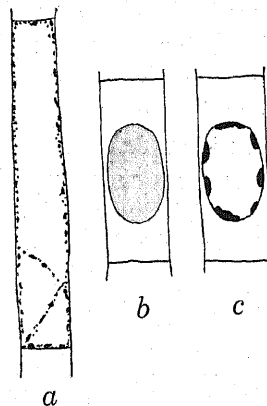


Abb. 2. a. Körnchenfärbung im wandständigen Plasma (Neutralrot). b-c: Vitalfärbung des Tonoplasten. Erklärung im Text.

färbt, die Vacuolen dagegen nicht färbt. Der Umstand, daß auch hier vor allem die Zellmembran sich färbt, darf als Bestätigung der STRUGGER'schen Beobachtungen angesehen werden, da *Basidiobolus* während seiner Entwicklung das Milieu ansäuert (BECKER u. SKUPIEŃSKI). Andererseits ist die Tatsache beachtenswert, daß in dem plasmatischen Wandbeleg der Farbstoff in Form von Körnchen ausgefällt wird, was wahrscheinlich irgendwelchen besonderen Eigenschaften des Wandbelegs zuzuschreiben ist, der in gewisse Reaktion mit dem Farbstoff eingeht. GICKLHORN (1929) stellte fest, daß wenn im Plasma „somatoide Kristalle“ sich färben, die Vacuole sich vital nicht färbt. Auch im Laufe der Desintegration des mit Neutralrot gefärbten Protoplasten von *Allium* treten im wandständigen Plasma rotgefärbte Kugeln und strauchartige Bildungen auf, worauf die Vacuole allmählich sich entfärbt (BECKER u. BECKEROWA, 1934).

Mit plasmolysierten und tonoplastenhaltigen (Abb. 1b) Zellen ergibt die Vitalfärbung ebenfalls kein nennenswertes Resultat. Der Inhalt der Tonoplasten färbt sich nicht, nur die Zellmembran. In einigen Ausnahmefällen färbte sich mit Neutralrot auch der Inhalt des Tonoplasten. Binnen kurzem verschwindet jedoch der Farbstoff rasch aus dem Terrain der Vacuole und wird in Form blutroter Kugeln unter der Oberfläche des Tonoplasten ausgefällt (Abb. 2b-c). Diese Beobachtungen bilden eine vortreffliche Ergänzung und Erklärung der Tatsache, daß im Cytoplasma kleine Körnchen sich färben.

Zusammenfassend stellen wir fest, daß obwohl die Vitalfärbung die Anwesenheit typischer Vacuolen in den „leeren“ Zellen nicht ergab, dies unserer These von der Gegenwart einer Plasmahaut unter der Zellmembran nicht widerspricht. Ja, die Vitalfärbung scheint dafür zu sprechen, daß der in den „leeren“ Zellen zurückgebliebene Wandbeleg gewisse spezielle Eigenschaften besitzt, da er sehr leicht in näher unbekannte Verbindungen mit dem Vitalfarbstoff eingeht. Der gemeinsam mit Dr. F. X. SKUPIEŃSKI unternommene Versuch *Basidiobolus* auf Nährlösungen, die sauren Farbstoffe enthalten, zu ziehen ergab ebenfalls kein deutliches Resultat. Eosin und Erythrosin scheinen jedoch in den „leeren“ Zellen die Zentralvacuole zu färben.

### c. Mikrodissektion

Die Mikrodissektion von *Basidiobolus* führten wir mit dem PÉTERFI'schen Mikromanipulator unter Zuhilfenahme hackenförmiger Nadeln aus. Das Objekt war entweder auf einer Agar-Unterlage befestigt, bzw. lag direkt in einem hängenden Tropfen.

Wenn man irgendeine „leere“ Zelle mit der Nadel des Mikromanipulators ansticht, erfolgt in den benachbarten Zellen eine

charakteristische Einhöhlung der Zellmembranen nach innen, was auf das Vorhandensein eines Turgorunterschiedes zwischen den verletzten und normalen Elementen hinweist (Abb. 3a). Mit zwei Nadeln manipulierend kann man die Terminalzelle vom Faden abtrennen; die Basalwand der Terminalzelle höhlt sich in diesem Falle nach außen aus (Abb. 3b). Nicht so leicht gelingt die Isolierung einer „leeren“ Zelle; auch in diesem Falle erfahren die Querwände eine ähnliche Aushöhlung (Abb. 3c). Aus den erwähnten mikrochirurgischen Beobachtungen folgt, daß die plasmaarmen Zellen, die bisher als leere Kammern galten, durch eine gewisse Turgeszenz sich auszeichnen. Außer den geschilderten experimentellen Beweisen für die Anwesenheit plasmatischer Häutchen in den beim Schrittwachstum abgegliederten Zellen,

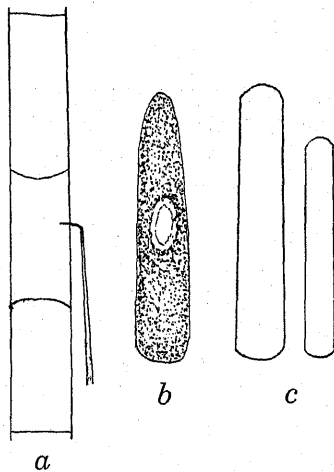


Abb. 3. a. Kernlose Zelle mit der Nadel des Mikromanipulators angestochen. b-c: isolierte kernlose Zellen und Terminalzelle.

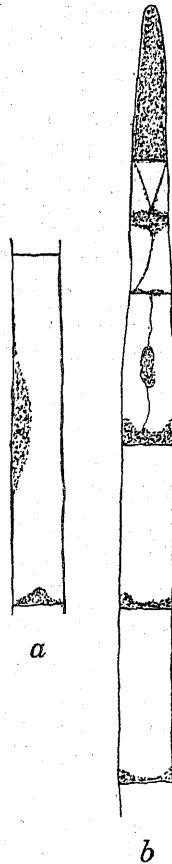


Abb. 4. Größere, in den kernlosen Zellen zurückgebliebene Plasmastücke.

können die Plasmahäutchen auch mittels gewöhnlicher Vitalbeobachtung festgestellt werden. Von besonderer Beweiskraft sind diejenigen Ausnahmefälle, wo größere Mengen von Plasma zurückbleiben. Einen derartigen Faden wiedergibt die schematische Abb. 4.

Wenn in der kernlosen Zelle eine größere Menge Plasma zurückbleibt, verschwindet immer der körnige braune Inhalt desselben (Glykogen nach RACIBORSKI) nach kurzer Zeit und verwandelt sich in eine strukturlose, hyaline Masse, die meistens mit dem Wandbeleg



zusammenfließt. Auf den ersten Blick scheint eine derartige Zelle frei von Plasma, erst bei näherer Untersuchung kommt der hyaloplasmatische Wandbeleg zum Vorschein.

\* \* \*

In Anbetracht der geschilderten Tatsachen müssen wir unsere Anschauungen über das Wesen des Schrittwachstums, wenigstens was *Basidiobolus* anbelangt, einer Revision unterziehen. Im Lichte der geschilderten Ergebnisse erweist sich das Schrittwachstum als Abgliederungsprozeß kernloser, plasmaarmer Zellen. Wir können diesen Prozeß mit der letzters von KÜSTER (1936) geschilderten Bildung von kernlosen Zellen bei *Plagiospermum* u. anderen Organismen vergleichen. Der Unterschied besteht einzig darin, daß in dem von KÜSTER geschilderten Falle die Kontraktion und Verlagerung des Plasmas mit dem Wachstum der Zelle nicht verknüpft sind. Freilich läßt sich auch bei *Basidiobolus* das Wachstum des Fadens experimentell unterbrechen, ohne daß dabei die Kontraktion und Verlagerung des Plasmas und die Abgliederung der kernlosen Zellen gestört wird (BECKER u. SKUPIEŃSKI).

Die Mikrodissektion und die Plasmolyse bewiesen, daß die kernlosen, plasmaarmen Zellen eine gewisse Turgeszenz aufweisen. Die Richtigkeit derjenigen Anschauungen, die das Schrittwachstum von *Basidiobolus* als Beispiel für Wachstum ohne Anteilnahme des Turgors angeben, muß demnach bezweifelt werden. Das Austreten des Wassers aus der basalen Vacuole, nach der Auswanderung des Plasmas in den Terminalteil des Fadens, ist gar nicht so offensichtlich, wie RACIBORSKI es darzustellen versucht. Wir müssen immerhin damit rechnen, daß das an der Membran zurückbleibende Cytoplasma andere Eigenschaften als die Plasmahaut in der Terminalzelle besitzen kann (vgl. Vitalfärbung). KÜSTER (1929) behandelt charakteristischerweise das Schrittwachstum im Kapitel „Anomale Plasmolysen“. Bekanntlich erfahren bei der Plasmolyse die oberflächlichen Schichten des Protoplasmas eine Zerreißen (vgl. Plasmoschise) und die an der Membran zurückbleibenden Plasmastücke weisen bereits ganz andere Eigenschaften auf. Es ist daher nicht ausgeschlossen, daß auch während der Systole bei *Basidiobolus* in der plasmatischen Haut, die an der Membran zurückbleibt, weitgehende Veränderungen stattfinden u.a. auch Änderungen in der Durchlässigkeit. Ich bin allerdings weit davon entfernt zu behaupten, daß der Zellsaft von der Plasmahaut ganz befreit, sich ganz bestimmt mit der Umgebung vereinigt.

Die hier erörterten Fragen knüpfen an derartige Probleme wie die Oosporenbildung oder Zoosporenbildung bei *Saprolegnia* an. Das



Terrain der Sporenbildung liegt im Oogonium bzw. Zoosporangium gleich unter der Membran; das Plasma kontrahiert sich allmählich und sammelt sich auf dem Terrain der Sporen an. BERTHOLD glaubte (1886), daß der zwischen den Sporenanlagen liegende Wandbeleg sich langsam, in dem Maße als die Sporen gebildet werden, von der Membran lostrennt. Auf Grund der Beobachtungen ROTHERT's (1890) und der eigenen (BECKER, 1934) kann ich jedoch feststellen, daß der Wandbeleg während der ganzen Zeit der Sporenbildung im Oogonium zurückbleibt und, daß erst kurz nach Abschluß der Kontraktion der Wandbeleg momentan auseinanderreißt, so daß der im Oogonium befindliche Saft herausfließt. Dieser Vorgang läßt sich am besten an vitalgefärbten Oogonien beobachten (BECKER, 1934). Ähnliche Zellsäftausscheidungen beobachteten BERTHOLD, HARTOG und ROTHERT in den Zoosporangien von *Saprolegnia*. Die in der Kultur vorhandenen Bakterien sammeln sich dann rings eines derartigen Zoosporangiums an. HARTOG (1887) stellte fest, daß während der Zoosporenentwicklung ein Moment eintritt, wo das Plasma plötzlich mit Eosin sich färbt, obwohl es bisher sich nicht gefärbt hat. HARTOG nimmt ebenso wie BERTHOLD an, daß in diesem eben Momente die Hautschicht, die das Zoosporangium auskleidet, zugrunde geht. In den weiteren Erörterungen gelangt HARTOG zu dem Schlusse, daß später sowohl die Hautschicht, wie die Vacuolenwand sich de novo bilden. ROTHERT, der HARTOG's Untersuchungen überprüfte, bestätigte indessen HARTOG's Beobachtungen nicht. ROTHERT behauptet, daß kein Grund vorliegt um das vollständige Verschwinden der Hautschicht anzunehmen, wie HARTOG und BERTHOLD es tun. Es genügt eine lokale Zerreißung des Wandbelegs, damit der Ausfluß des Zellsaftes und die durch HARTOG geschilderte Färbung eintreten.

Die Ansicht ROTHERT's scheint uns vollkommen berechtigt. Eine Bekräftigung seiner Ansicht dürfte ROTHERT in den letzten Studien KÜSTER's über das Auftreten von Löchern im Protoplasma von *Codium* finden. Aus allem dem folgt, daß obwohl RACIBORSKI gar keine konkreten Beweise für das Austreten des Saftes aus der Vacuole der „leeren“ Zellen von *Basidiobolus* anführt, wir mit dieser Möglichkeit dennoch rechnen müssen. Freilich kann der Ausfluß des Zellsaftes auch nach der Abgliederung der Terminalzelle eintreten, ähnlich wie in den Oogonien von *Saprolegnia*, wo er oft erst nach der Kontraktion der Sporen erfolgt.

Wie daraus zu ersehen, ist die Frage, ob das Wachstum von *Basidiobolus* ohne Anteilnahme des osmotischen Turgors oder mit dessen Anteil erfolgt, gar nicht so einfach und kann angesichts des gegenwärtigen Standes der Dinge nicht mit voller Bestimmtheit

beantwortet werden. Meiner Meinung nach hängt es ganz vom Zufall ab, ob die Plasmahaut unverletzt bleibt oder an gewissen Stellen zerreißt. Beim Schrittwachstum sind vielleicht hie und da beide Eventualitäten möglich. Was unsere eigene Beobachtungen anbetrifft, so konnten wir, obwohl wir die mit Bakterien verunreinigten Kulturen genau überprüften, eine Ansammlung der Bakterien rings um die Basalvacuole nicht beobachten. Vitalfärbungen ergeben ebenfalls—wie wir gesehen haben—kein klares Resultat. Es bestehen daher gar keine experimentellen Beweise dafür, daß der Zellsaft der „leeren“ Zellen bzw. der Basalvacuole nach außen austritt. Immerhin rechnen wir auch mit dieser Möglichkeit und nehmen an, daß unverletzte Plasmahäutchen, wie auf Abb. 1 dargestellten, manchmal sekundär nach der Restitution der Lücken und Löcher entstehen können. Die Möglichkeit einer derartigen Restitution bei den Siphoneen wird von KÜSTER angenommen (1933, 45; näheres über diese Frage s. KÜSTER 1933 u. 1935).

Das Vorhandensein eines stabilen osmotischen Druckes während des Wachstums von *Basidiobolus* ist in Anbetracht dessen, daß beim Membranwachstum vor allem der Kolloiddruck des Plasmas die Hauptrolle spielen kann, vielleicht sogar von zweitrangiger Bedeutung (Lit. vgl. HEYN, 1931 und STRUGGER, 1934).

Wenn auch unsere eigenen Beobachtungen uns ein sicheres Urteil in der berührten Frage nicht gestatten, ist die Feststellung der Gegenwart des Plasmas in den „leeren“ Zellen für gewisse Erscheinungen des Membranwachstums nicht ganz ohne Bedeutung. Ich denke dabei vor allem an das Wachstum und die Differenzierung des Exospors in den Makrosporen von *Selaginella*, welche seit FITTING als klassisches Beispiel für Membranbildung und Membranwachstum ohne Teilnahme des Plasmas bekannt sind (TISCHLER, 1934; KÜSTER, 1935).

Die Plasmakontraktion in den Makrosporen von *Selaginella* ist hinsichtlich ihres Mechanismus sicherlich der Plasmakontraktion in den *Basidiobolus*-Fäden ähnlich. Wir machten bereits früher darauf aufmerksam, daß man bei *Basidiobolus* das Wachstum des Fadens aufhalten kann, ohne daß dabei die Plasmakontraktion und die Abgliederung der „leeren“ Zellen unterbrochen wird (BECKER u. SKUPIŃSKI). Ich bin geneigt anzunehmen, daß auch in den Makrosporen von *Selaginella* neben dem Exosporium ein Wandbeleg zurückbleibt, der später die Wachstums- und Entwicklungsprozesse dieser Membran leitet. Nähere Untersuchungen in dieser Hinsicht wären jedoch erwünscht.

Es bleibt zu fragen, wieso es möglich war, daß RACIBORSKI, der das Schrittwachstum von *Basidiobolus* so genau verfolgte, die Anwesenheit des Plasmas in den abgegliederten Zellen übersah, trotzdem

es sogar mit schwachen Objektiven sich leicht feststellen läßt. In Gesprächen mit den gewesenen Assistenten und Mitarbeitern von RACIBORSKI, den Herrn Prof. Dr. J. KOŁODZIEJCZYK und Oberst Dr. T. RÓŻYCKI, erfuhr ich, daß RACIBORSKI in den späteren Jahren sich der Unzulänglichkeit seiner Beobachtungen in diesem Falle bewußt war, und wiederholt seiner Meinung Ausdruck gab, daß das Schrittwachstum noch eine nähere Analyse erfordert. Leider erlaubte der frühzeitige Tod diesem genialen Botaniker nicht seine Arbeit in dieser Hinsicht zu Ende zu führen.

### Zusammenfassung

Auf Grund der Untersuchungen über das Schrittwachstum von *Basidiobolus ranarum* wurde festgestellt, daß das Schrittwachstum auf einer rhythmischen Kontraktion des Plasmas in dem Terminalteil der Hyphe und der Abgliederung kernloser, plasmaarmer Zellen beruht. Es wurde auf andere Fälle ähnlicher Plasmakontraktion, sowie auf die Möglichkeit des Zurückbleibens dünner Hyaloplasmaschichten an der verlassenen Membran hingewiesen.

### Literatur

- Becker, W. A. 1934. Experimentelle Untersuchungen über die Vitalfärbung sich teilender Zellen. III. Mitt. Weitere Studien über die Cyto-kinese. Acta Soc. Bot. Poloniae, 11: 139.
- und Beckerowa, Z. 1934. Über einige Desintegrationserscheinungen des Protoplasten. Acta Soc. Bot. Poloniae, 11: 367.
- et Skupieński, F. X. 1935. Observations protoplasmiques vitales sur *Basidiobolus ranarum*. C. Rend. Acad. Sci., 200: 1620.
- Gicklhorn, J. 1929. Kristalline Farbstoffspeicherung im Protoplasma und Zellsaft pflanzlicher Zellen nach vitaler Färbung. Protoplasma, 7: 341.
- Hartog, M. M. 1887. On the formation and liberation of the zoospores in the Saprolegniaeae. Quart. Journ. Micr. Sc. 417.
- Heyn, A. N. J. 1931. Der Mechanismus der Zellstreckung. Rec. Trav. Bot. Neerlandais, 28: 113.
- Hofmeister, W. 1867. Die Lehre von der Pflanzenzelle. 1: 404 S.
- Kniep, H. 1928. Die Sexualität der niederen Pflanzen. Jena.
- Küster, E. 1929. Pathologie der Pflanzenzelle I. Berlin.
- 1932. Protoplasmabewegungen in zentrifugierten Zellen. Ber. Oberhess. Ges. Natur- u. Heilkd. Gießen, 15.
- 1933. Dellen und Löcher im Protoplasma lebender Pflanzenzelle. Protoplasma, 19: 335.
- 1935. Die Pflanzenzelle. 672 S.
- 1936. Über kernlose Zellen. Cytologia, 7: 246.
- Laibach, F. 1928. Über Zellfusionen bei Pilzen. Planta, 5: 340.
- Pfitzer, E. 1872. Ein neuer Algenparasit aus der Ordnung der Phycomycetes. Monats-Ber. Acad. Wiss. Berlin. 379, Zit. nach KÜSTER, 1935.
- Pringsheim, N. 1873. Weitere Nachträge zur Morphologie und Systematik der Saprolegnien. Jahrb. wiss. Bot., 9: 191.

- Raciborski, M. 1907. Über Schrittwachstum der Zelle. Bull. Int. Acad. Sci. Cracovie. Cl. Math. Sci. et Nat., 898.
- Rothert, W. 1890. Die Entwicklung der Sporangien bei den Saprolegnieen. Ein Beitrag zur Kenntnis der freien Zellbildung. Beitr. Biol. der Pfl., 5: 291.
- Strasburger, E. 1882. Über den Bau und das Wachstum der Zellhäute. Jena.
- Strugger, S. 1934. Beiträge zur Physiologie des Wachstums I. Zur protoplasma-physiologischen Kausalanalyse des Streckungswachstums. Jahrb. wiss. Bot., 79: 406.
- 1935. Beiträge zur Gewebephysiologie der Wurzel. Zur Analyse und Methodik der Vitalfärbung pflanzlicher Zellen mit Neutralrot. Protoplasma, 24: 108.
- Tischler, G. 1934. Allgemeine Pflanzenkaryologie. I. Der Ruhekern. Berlin.
- Wieben, M. 1927. Die Infektion, Myzelüberwinterung und die Kopulation bei Exoasceen. Forsch. Geb. d. Pflanzenkrankh., 3: 139. Zit. nach KÜSTER, 1935.
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## Relationship Between Various Chromosomal Changes in *Drosophila melanogaster*

By

**M. Demerec**

Carnegie Institution of Washington, Cold Spring Harbor,  
N. Y., U. S. A.

In order to understand the mechanism instrumental in producing various genetic changes such as gene mutations, lethals, inversions, and translocations it is essential to have a knowledge of the interrelationship of these changes. It is particularly important to know whether or not any of them occur simultaneously and if they do, the frequency with which they occur. Coincident occurrence of certain changes would place the responsibility for their origin on the same mechanism and would simplify the problem if such coincidence were found to be a rule.

In the course of our studies of various genetic changes an extensive collection of small deficiencies, gene mutations, translocations, and inversions has been accumulated. This material has been studied cytologically and genetically and the results of these studies will be reported here.

By X-ray treatment a number of lethal and visible changes were induced in various X-chromosome loci. To detect these changes females carrying several recessive characters were mated with treated males. If any character carried by their mothers appeared in the  $F_1$  females this indicated that a change in that particular locus was induced by the treatment. Thus, if *ec ct v g f* females were mated with + treated males, the appearance of *ct* in one of the  $F_1$  females would indicate that a change in *ct* was produced in the sperm of a treated male. All lethal changes obtained in the loci selected for observation were kept in stocks but only a few of the visible changes were saved. This material has been collected from experiments conducted since 1933. The dosage in various treatments varied between 2500 and 3000 r-units.

In this manner a collection of about 80 lethals affecting visible loci of the X-chromosomes has been accumulated. Salivary chromosomes of 61 of these lethals and of 15 visibles obtained in the same experiments were examined for chromosomal aberrations. In cases where such an aberration was found it has been determined through salivary chromosome analysis whether or not the chromosomal break

is located in the region where the lethal or visible change occurred. A summary of the results is given in Table 1. It is evident from these data that out of a total of 61 lethal changes studied, in 26 or 42.6 per cent of the cases a chromosomal aberration occurred in the

Table 1. Summary of data showing the frequency of coincidence between various genetic changes induced by X-ray treatment of about 2500 to 3000 r-units. (C. A. = chromosomal aberration; In = inversion; T = translocation)

Locus involved	Total no. of cases	Without C. A.	Change and C. A. coinciding		Change and C. A. not coinciding	
			In	T	In	T
<i>Lethal changes</i>						
left of y	1	1	—	—	—	—
y ac	1	1	—	—	—	—
pn w	1	1	—	—	—	—
w	3	2	—	1	—	—
w N	1	—	—	1	—	—
N	6	2	1	3	—	—
ct	16	6	5	5	—	—
tlz	1	1	—	—	—	—
v	1	1	—	—	—	—
m	1	1	—	—	—	—
m dy	3	2	—	1	—	—
M wys	1	1	—	—	—	—
wy	1	—	—	1	—	—
g	2	2	—	—	—	—
g ty	4	4	—	—	—	—
f	7	5	—	2	—	—
B	8	4	2	1	1	—
Bx	3	1	1	—	1	—
Total	61	35	9	15	2	—
<i>Visible changes</i>						
sc-cv	1	1	—	—	—	—
y	1	1	—	—	—	—
w	17	17	—	—	—	—
ec	1	1	—	—	—	—
v	1	1	—	—	—	—
m	3	3	—	—	—	—
Brs	1	1	—	—	—	—
g	1	—	—	—	1	—
r	1	1	—	—	—	—
f	3	3	—	—	—	—
Total	30	29	—	—	1	—

same chromosome with the lethal change. The significant fact brought out here is the high degree of coincidence between the two types of changes. In cases where both changes occurred in the same chromosome, one breakage point of the chromosomal aberration coincided with the region where the lethal change took place in 92.3 per cent of the times. This extremely high coincidence shows that the same mechanism must be responsible for the origin of both types of changes, viz. of lethals and chromosomal aberrations, at least in cases where they occurred together.



The situation seems to be different in the case of visible changes. These changes were accumulated in the same or similar experiments as lethal changes. The X-ray dosage applied in producing these changes was similar to that used in producing lethals. Only one out of the 30 visibles investigated carried a chromosomal aberration (Table 1). In this one case, moreover, neither of the breakage points of the aberration coincided with the visible change. In 15 of these cases the analysis was made by studying salivary chromosomes and in the other 15 cases by the genetic method of linkage relation studies.

A similar relationship between induced lethals and chromosomal aberrations is indicated in the results of another experiment in which 100 treated sperms, taken at random, were tested for various changes. The wild-type males were treated with about 2500 r-units and mated to *sc ec cv ct v g f* females which carried markers almost throughout the whole length of the X-chromosome. Aberrations involving the X-chromosome were identified through reduction in crossing-over and lethals were identified through non-appearance of wild-type males. The following results were obtained:

Total number of tests .....	100
Lethals .....	11
Lethals and chromosomal aberrations .....	5
Chromosomal aberrations .....	5
Visible changes .....	2

In this experiment, also, a lethal change and a chromosomal aberration occurred in the same chromosome in a high proportion of cases. Though the cytological studies have not been made, it is justifiable to assume in this case that a large proportion of these changes coincided.

When this evidence became available it was interesting to find out the frequency of chromosomal aberration among spontaneous lethals. In order to do that, 45 spontaneous lethals occurring in males of various wild type stocks were collected by the CIB method. During this work it was discovered that the Florida wild stock carried a recessive factor in the second chromosome which increases the natural rate of mutability in this stock (Demerec, 1937). This factor is effective early in the embryonic development and thus if a lethal originates in its presence in a male, several sperms will carry it. In routine tests such a lethal change may be counted as several lethals. Special precautions were taken to be sure that all lethals used in these tests were of independent origin. The results of genetic tests show that none of the 45 spontaneous lethals were connected with chromosomal aberrations. Oliver (1932) made a similar study



with 10 spontaneous lethals and Sakharov (1936) with 25 lethals. They obtained the same results. Thus tests are available for 80 spontaneous lethals showing no chromosomal aberration coincident with the lethal change.

Available data show that spontaneous lethals are independent of chromosomal aberrations while the lethals induced by X-ray treatment are frequently connected with chromosome breaks. In Table 2

**Table 2.** Frequency of chromosomal abnormalities (C. A.) among lethals obtained from treatments with different X-ray dosages

Treatment r-units	Our Data			Oliver's Data		
	total	with C. A.	per cent	total	with C. A.	per cent
Control	—	—	—	10	—	—
385	—	—	—	57	3	5.3
770	—	—	—	72	9	12.5
1400	81	11	13.6	—	—	—
1540	—	—	—	55	4	7.3
2500	16	5	31.3	—	—	—
3000	57	23	40.3	—	—	—
3180	—	—	—	61	15	24.6
6160	—	—	—	70	24	34.3

data are given on the frequency of chromosomal aberrations in the material treated with different dosages. Although there is a discrepancy in the per cent of aberrations obtained by Oliver and those obtained in my experiments, it is evident from the data that the frequency of chromosomal aberrations increases rapidly with the increased dosage.

A large amount of data is available showing the relation between the X-ray dosage and the frequency of lethals. In only one case (Oliver 1932) were these lethals studied further and the proportion of chromosomal abnormalities occurring among them was determined. Oliver's data are given in Table 3 and shown graphically in figure 1,

**Table 3.** Oliver's (1932) data on the frequency of various kinds of lethals induced by X-rays. (C. A. = Chromosomal aberration)

Dosage	Cultures	Lethals					
		Total		Without C. A.		With C. A.	
		Number	per cent	Number	per cent	Number	per cent
Control	4033	10	0.24	10	0.24	—	—
385	4016	57	1.42	54	1.34	3	0.08
770	2231	72	3.23	63	2.83	9	0.40
1540	1144	55	4.90	51	4.55	4	0.35
3080	618	61	9.87	46	7.44	15	2.43
6160	435	70	16.09	46	10.47	24	5.52

It is evident from these data that the dosage-frequency curve for all lethals is a straight line. Such a curve, however, slopes down for lethals without chromosomal aberrations and slopes up for lethals with chromosomal aberrations.

The significance of this observation will be discussed later.

Random lethals dealt with in all experiments on the mutation frequency have not been investigated cytologically and it is not directly known whether or not they are deficiencies. There is ample evidence, however, to show that lethals affecting known loci are deficiencies. We studied cytologically lethals affecting the following loci: yellow-achaete, prune, white, facet, cut, tan, lozenge, vermilion, miniature, dusky, wavy, sable, garnet, tiny, forked and beadex. In every case they were found to be deficiencies and, moreover, mostly deficiencies for more than one salivary band, and presumably for more than one locus. It seems justifiable to assume that random lethals are similar to lethals of visible loci, and that they are also deficiencies. There is no doubt that among random lethals there are many which affect visible loci but they have not been detected as such since special tests are required to do that. Estimates on the number of loci per chromosome supported by counts of salivary chromosome bands indicate that there are at least ten times as many loci in the X-chromosome as there are known visible changes. It is undoubtedly true that some of these as yet unknown loci will be identified after more new mutations are found. Since new mutations are becoming scarcer and scarcer, in spite of greatly enlarged work with *Drosophila*, it seems very probable that there are many loci in which changes (mutations) show no visible morphological effect. If the estimate of the total number of loci is correct, it appears probable that the majority of loci are of a phenotypically non-detectable type. It is equally probable that deficiencies of some of these loci will have a lethal effect. The groups of random lethals, therefore, may be composed, in part, of deficiencies affecting visible loci and, in a larger part, of deficiencies affecting non-detectable loci. There might be

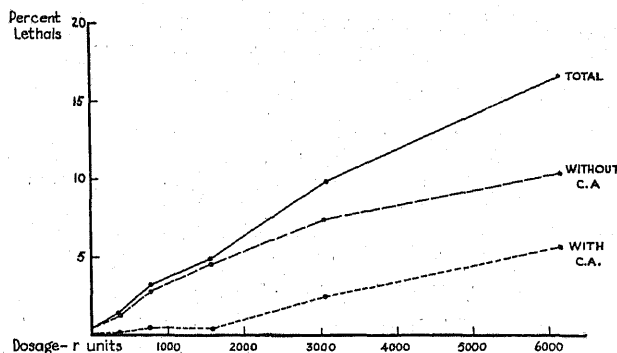


Fig. 1. Curves showing incidence of chromosomal aberrations among X-chromosome lethals induced by various X-ray dosages. (Oliver's data, 1932).

some lethals, however, which are not due to deficiencies but to gene changes having a lethal effect. The relative proportion of these two types of lethals will have to be determined through study of salivary chromosomes. This is a laborious process, especially when dealing with deficiencies affecting very fine bands.

In a recent paper, Sakharov (1936) states that he examined salivary chromosomes of heterozygous females of 25 X-chromosome lethals without finding any deficiencies. A similar work is being carried on at this laboratory by Dr. B. Slizynski with results different from those obtained by Sakharov. Of 13 spontaneous lethals which have been carefully studied, at least half have detectable deficiencies. This work is still in progress. Evidence obtained so far is in favor of the contention outlined above.

### Discussion

From the data presented in this paper it is evident that when a lethal and a chromosomal aberration occur in the same chromosome, one breakage point of the chromosomal aberration is at the place where the lethal change is located in a great majority of cases (92 per cent). Since such a coincidence cannot be expected if these two changes were occurring at random, it is evident that both of them must be induced by the same mechanism.

The data are also ample to show that chromosomal aberrations do not occur with equal frequency among lethals induced by different factors. No chromosomal aberration was found among 80 spontaneous lethals studied by Oliver (1932), Sakharov (1936), and myself, while almost fifty per cent of these induced by the 3000 r-units X-ray treatment carried chromosomal aberrations. The frequency of these aberrations increases with the X-ray dosage applied. All lethals are not connected with chromosomal aberrations, nor do all chromosomal aberrations carry lethals even at higher dosages.

What is the mechanism which can account for such conditions? There is no doubt about the interrelation of these two types of changes, but at the same time there cannot be any doubt that the type of change responsible for the lethal effect may occur independently of a chromosomal aberration. Is there any certainty that the opposite is true? In order to give a plausible answer to this question it is necessary to consider the problem of lethal changes. It has been pointed out earlier that the majority of lethals are probably deficiencies. If that assumption is taken for granted, then the statement made previously may be restated as follows: deficiencies frequently coincide with chromosomal aberrations. Since our methods for

determining chromosomal aberrations are reliable, it can be stated with certainty that a deficiency may occur without a chromosomal aberration. Such an independence in origin, however, may not hold true for chromosomal aberrations since they may regularly be connected with deficiencies undetectable by our ordinary methods. It has been shown (Demerec and Hoover, 1936) that a homozygous deficiency affecting as many as four bands may be viable and show no morphological effect on the organism. Such a viable deficiency can be detected in salivary chromosomes only. If it affects one light band or a few of them and is, in addition, connected with a chromosomal abnormality, it may be physically impossible with methods available at present to detect it at all. The possibility is not excluded, therefore, that such chromosomal aberrations as inversions and translocations not showing a lethal effect are connected with short deficiencies at their breakage points.

Let us now see if it would be of any help toward the solution of our problem if an assumption were made that chromosomal breakages are usually connected with deficiencies. It must be made clear at this point that I am considering here small deficiencies affecting only a few bands, and that as a rule such deficiencies, when they affect more than one band, are produced by some sort of block change. Such a block change is no doubt produced regularly through a chemical process, though the possibility is not excluded that it may be sometimes produced by the pinching out of a small section of a chromosome. If we assume that breakages, as a rule, are connected with small deficiencies, it would seem probable that these deficiencies might be responsible for the breakages, viz. at a spot where such a deficiency is produced a chromosome is either regularly or frequently broken. Because of the attraction between broken parts, broken chromosomes have a tendency to unite again thus showing a deficiency only. If, however, breaks should occur at more than one place in a chromosomal complex, subsequent fusion of broken ends may produce various chromosomal aberrations such as inversions if two breaks should occur in the same chromosome, and translocations if they should occur in different chromosomes. It would be expected then, that when the frequency of deficiencies is low they would not be connected with chromosomal aberrations, and the proportion of aberrations would increase with an increase in the frequency of deficiencies. This interpretation agrees with the observed facts.

None of the recessive visible gene mutations (induced by the same treatment which produced among lethals about 40 per cent of chromosomal aberrations) was connected with chromosomal aberrations. This suggests that visible gene mutations are different from

lethals. It makes it probable, also, that gene mutations, rather than gene deficiencies, are responsible for the majority of recessive visible changes.

### Summary

From a total of 61 lethals affecting known loci of the X-chromosome, and induced by X-ray treatment of 2500 to 3000 r-units, 26 or 42.6 per cent had chromosomal aberrations such as inversions and translocations. In 92.3 per cent of cases one breakage point of the chromosomal aberration coincided with the region where the lethal change took place.

Of 30 visible mutations induced by a similar treatment only one carried a chromosomal aberration. This, however, did not coincide with the region where visible change took place.

Data available on 80 spontaneous lethals show that none was connected with either an inversion or a translocation. Among lethals induced by X-ray treatment, the frequency of such chromosomal aberrations increases with the increase of dosage.

It has been determined by salivary chromosome studies that all investigated lethals affecting known loci are minute deficiencies. It is suggested that either all or a great majority of random lethals are also deficiencies, and that some of the small deficiencies may not have a lethal effect.

Coincidence between a breakage point of a chromosomal aberration and the place where a deficiency has occurred indicates that these two processes may be induced by the same mechanism. Chemical changes producing small deficiencies are responsible for breaks in chromosomes. Free ends produced by such breaks have a tendency to join again. If the frequency of breaks in a nucleus is low, as in case of spontaneous changes, chromosomal rearrangements have little chance to occur, but if it is high, as in case of high dosage X-ray treatment, the opportunity for the origin of chromosomal rearrangements (inversions and translocations) is also high.

### Literature cited

- Demerec, M., 1937. A mutability stimulating factor in the Florida stock of *Drosophila melanogaster*. *Genetics* 22: 190.
- Demerec, M. and M. E. Hoover, 1936. Three related X-chromosome deficiencies in *Drosophila*. *Jour. Hered.* 27: 206-212.
- Oliver, C. P. 1932. An analysis of the effect of varying the duration of X-ray treatment upon the frequency of mutations. *Ztschr. ind. Abst. u. Vererb.* 61: 447-488.
- Sakharov, V. V., 1936. On the specificity of the action of factors causing mutations. *Bul. Biol. et Med. Exp. (Russian)* 1: 202-203.

## Über die Atmungsfarbstoffe von *Paramecium*

Von

**Tadao Sato**

Zoologisches Institut, Kaiserl. Universität Tokyo

und

**Hiroshi Tamiya**

Botan. Inst., Kaiserl. Universität Tokyo und  
Tokugawa Biologisches Institut

Obwohl die spektroskopischen Untersuchungen über die intrazellulären Häminverbindungen, die im Mechanismus der aeroben Zellatmung eine hervorragende Rolle spielen, bereits an mannigfältigsten Organismen sowohl von tierischer als auch von pflanzlicher Herkunft mit viel Erfolg ausgeführt worden sind, blieb unsere diesbezügliche Kenntnis bei Urtieren bisher vollständig in blanko gelassen.<sup>1)</sup> Diese Lücke zu erfüllen war die Aufgabe vorliegender Arbeit, in welcher zunächst ein leicht in Masse kultivierbares Urtier, *Paramecium* (*caudatum*-Typ), als Versuchsobjekt herangezogen wurde. Ganz unerwartet standen wir dem Tatbestand gegenüber, daß dieser Organismus nicht nur einen eigenartigen Cytochromtyp aufweist, sondern auch eine ansehnliche Menge vom Hämoglobin enthält, dessen Anwesenheit zwar bei solch einem niedrigen, unizellularen Organismus noch nie nachgewiesen worden ist.

Zucht des Organismus geschah in einer Erdabkochung unter Zusatz des Heues, wobei in günstigen Fällen aus 1 Liter Kulturflüssigkeit schon nach 8- bis 10-tägiger Kultur eine zur direkten Spektroskopierung genügende Menge des Materials gewonnen werden konnte. Allerdings war dabei Verunreinigung durch *Colpidium*-Arten nicht zu vermeiden, die aber wegen ihrer bedeutend kleineren Körpergröße durch Kolieren mit Seidentuch größtenteils abgetrennt werden konnten. Die auf dem Koliertuch zurückbleibenden *Paramecien* wurden nach wiederholtem Waschen mit Wasser in dest. Wasser suspendiert und in einem langen Zylinderglas (von etwa 1 Liter Inhalt) bei 25°C stehen gelassen. In einigen Stunden sammelten sich die *Paramecien*, dank ihrem angeblichen „negativen Geotropismus“, meist an oberen Teil des Gefäßes, während *Colpidien*, wenn vorhanden, flockenartig an Boden sanken. Nach Beseitigung dieses *Colpidien*flockens durch Pippettierung wurde die Flüssigkeit zentrifugiert. Pro Liter von ursprünglicher Kulturlösung läßt sich auf diese Weise etwa 1–2 ccm von einer praktisch reinen *Paramecien*-

1) Vergl. z.B. DOFLEIN u. REICHENOW. 1927.



masse erhalten.<sup>1)</sup> Die gesammelten Zellen wurden ferner auf der Zentrifuge wiederholt mit dest. Wasser gewaschen, dick in demselben suspendiert, und dann in einem modifizierten THUNBERG-Rohr<sup>2)</sup> unter Anwendung eines Okularspektroskops nach ABBÉ spektroskopiert. Als Lichtquelle diente eine Punktlichtlampe („Wotanlampe“) von der Firma ZEISS.

Die unter verschiedenen Bedingungen beobachteten Bilder sind in der beiliegenden Figur schematisch wiedergegeben. Zuerst unter anaerobem Zustand (bei Evakuierung) kamen folgende Absorptionen zur Beobachtung:

- I. Ein schmales und verschwommenes Band an 613–603  $m\mu$ , mit einem Absorptionsmaximum an 608  $m\mu$ .
- II. Ein sehr breites und deutlich asymmetrisches Band an 585–549  $m\mu$ , wobei besonders im Bereich 557–549  $m\mu$  mit einem Maximum an 551  $m\mu$  stark absorbiert ist.
- III. Ein sehr verwaschenes Band an 527–519  $m\mu$  mit einem Maximum an ca. 523  $m\mu$ .

Bemerkt sei hier vorwegnehmend, daß die Absorption II aus zwei Bändern mit dem Maximum an ca. 555  $m\mu$  bzw. 551  $m\mu$  besteht, obwohl dieselben in Wirklichkeit nicht getrennt erkannt werden können.

Beim Schütteln der Suspension mit Luft oder bei Durchströmung des Sauerstoffs in die Suspension verschwinden alle obigen Streifen, und anstatt deren treten ein auffallend intensives schmales Band an 581  $m\mu$  (588–575  $m\mu$ ) und ein verschwommenes diffuses Band an 545  $m\mu$  (554–536  $m\mu$ ) hervor. Stellt man dann die Suspension ruhig, oder wird die Suspension plötzlich evakuiert, so werden diese beiden Streifen allmählich unsichtbar, und zugleich das Bild unter anaerobem Zustand wieder ganz reversibel hergestellt. Diese vollständige Reversibilität unter aerober bzw. anaerober Bedingung weist ohne Zweifel auf die „respiratorische“ Natur der Substanzen hin, zu welchen die in Betracht kommenden Streifen gehören. Manchmal und zwar bei schwach atmenden *Paramecien*zellen werden die anaeroben und die aeroben Streifen gleichzeitig beobachtet, wobei aber durch O<sub>2</sub>-Zugabe die ersteren zum gänzlichen Verschwinden und

1) Trotz mehrmaligem Waschen mit dest. Wasser dürfte hierbei die Möglichkeit etwaiger Beimengung der Bakterienzellen nicht ganz ausgeschlossen sein, zumal da die *Paramecien*, wie schon von mehreren Autoren dargetan, auf Kosten der beigemengten Bakterienzellen auskommen. Daß aber in unsere spektroskopische Befunde keine Unsicherheit wegen dieser Möglichkeit eingeschlichen sein kann, geht ohne weiteres daraus hervor, daß die spektroskopischen Bilder bei Bakterien, wie die umfangreichen systematischen Untersuchungen von TAMIYA u.a. (YAOI und TAMIYA 1928, TAMIYA and YAMAGUTCHI 1933, FUJITA 1934) zeigen, gründlich von denjenigen von unserem Versuchstier verschieden sind.

2) Vergl. H. TAMIYA u. Y. OGURA (1937).



die letzteren zur auffallenden Verstärkung gebracht werden.

Ein etwas anderes Bild findet man bei Zusatz von KCN (M/500–M/1000). Während das anaerobe Bild durch CN-Zusatz gar nicht modifiziert wird, bestehen hierbei bei Schütteln mit O<sub>2</sub> die Streifen

an 608, 551 und 523

m $\mu$ , neben den neu

auf tretenden aeroben

Bändern an 581 und

545 m $\mu$ , unverändert

fort, indem aber die

Absorption im Gelb-

grün sich zu 554–

549 m $\mu$ , ebenfalls mit

dem Maximum an

551 m $\mu$ , deutlich sch-

mälert. Das Auftre-

ten der Streifen an

581 und 545 m $\mu$  bei Schütteln mit Luft wird hierbei nicht nur nicht

verhindert, sondern sogar auffallend verdeutlicht. Übrigens erfolgen

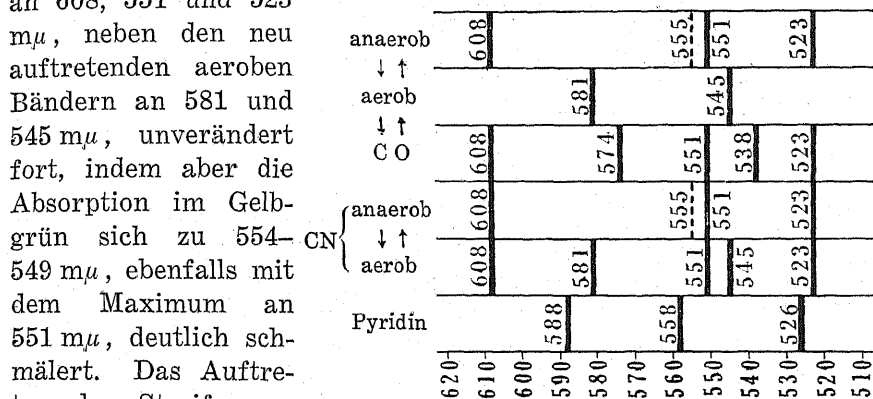
auch bei CN-Zugabe das Auftreten und das Verschwinden dieser

beiden Streifen unter aerober bzw. anaerober Bedingung vollkommen

reversibel, wie es in der Figur dargestellt ist.

Einem noch merkwürdigeren Tatbestand stößt man bei Durchströmung des Kohlenoxyds in die Suspension. Es tritt nämlich ein neuer intensiver Streifen an 574 m $\mu$  (579–568 m $\mu$ ) hervor, und gleichzeitig erweitert sich die Absorption im Grün zu 540–519 m $\mu$ . Diese letztere Absorption scheint dabei aus zwei Bändern zu bestehen, für deren Absorptionsmaximum wohl die Werte 538 m $\mu$  bzw. 523 m $\mu$  angenommen werden dürften. Andererseits tritt hierbei, wie es bei CN-Zugabe in aerobem Zustand der Fall war, die Schmälerung des Bandes im Gelbgrün zu 553–549 m $\mu$  (mit dem Maximum an 551 m $\mu$ ) ein, während der Streifen an 608 m $\mu$  (613–603 m $\mu$ ) gar keine Modifizierung erfährt. Bei nachträglicher Durchleitung des Sauerstoffs geht das ganze spektrale Bild sofort wieder zu demjenigen zurück, welches man unter aerober Bedingung beobachtet hat. Auch bei längerer Evakuierung nach der Sättigung der Suspension mit CO wird der Einfluß von CO beseitigt, sodaß die Suspension schließlich dasselbe Bild wie unter anaerober Bedingung aufweist.

Gestützt auf die Tatsache, daß bei Zugabe von CO oder bei Schütteln mit O<sub>2</sub> unter CN-Zusatz eine unverkennbare Schmälerung der Absorption im Gelbgrün stattfindet, ist es zu schließen, daß die unter anaerobem Zustand beobachtete Absorption II aus folgenden zwei Bändern besteht:



- (1) Ein intensives und schmales Band an 553–549  $m\mu$  mit einem Maximum an 551  $m\mu$ .
- (2) Ein diffuses und asymmetrisches Band mit dem Maximum an etwa 555  $m\mu$  und der langwelligen Grenze an ungefähr 585  $m\mu$ . Die kurzwellige Grenze dieses Bandes ist wegen des Verschmelzens mit dem Streifen 1 nicht zu bestimmen.

Beim Überblick über das Verhalten der einzelnen Streifen besteht zunächst kein Zweifel darüber, daß der Streifen an 608  $m\mu$  (613–603  $m\mu$ ) der  $\alpha$ -Komponente, und die Streifen an 551  $m\mu$  (553–549  $m\mu$ ) und an 523  $m\mu$  (527–519  $m\mu$ ) der  $c$ -Komponente des Cytochroms zukommen.<sup>1)</sup> Was nun die übrigen Streifen anbelangt, so kennzeichnen sie sich von denjenigen der gewöhnlichen Zellhämone dadurch, daß sie unter anaerober Bedingung undeutlicher und bei Zugabe von  $O_2$  oder CO mit einer sichtlichen Lageverschiebung auffallend intensiver werden.<sup>2)</sup> Dieses Verhalten und die Lage der erwähnten Absorptionen bei  $O_2$ - und CO-Zugabe sprechen befriedigend dafür, daß sie einem Hämoglobin und dessen Ferro- $O_2$ - bzw. -CO-Verbindung angehören. Erwartungsgemäß kam bei Zusatz von Chinon ein Streifen an 633  $m\mu$  (640–625  $m\mu$ ) zum Vorschein, der auf die Bildung von Methämoglobin hinweist.

Um unsere Beweisgründe über die Hämoglobinnatur der in Frage kommenden Substanz übersichtlich zu machen, wollen wir unten unsere spektroskopischen Befunde denjenigen von gewöhnlichem Hämoglobin gegenüberstellen.

	<i>Paramecium</i> -Hämoglobin	Menschen-Hämoglobin
Desoxygenierte Form	585–549 (?) $m\mu$ unscharf begrenzt, mit einem Maximum an etwa 555 $m\mu$ .	570–546 $m\mu$ unscharf begrenzt, mit einem Maximum an etwa 555 $m\mu$ .
Oxygenierte Form	581 $m\mu$ , 545 $m\mu$ .	577 $m\mu$ , 542 $m\mu$ .
CO-Verbindung	574 $m\mu$ , 538 $m\mu$ .	571 $m\mu$ , 538 $m\mu$ .
Methämoglobin	633 $m\mu$ .	633 $m\mu$ .

Daß die Lage der Absorptionen von Hämoglobin und seinen Derivaten je nach ihrer Provenienz um einige  $m\mu$  verschieden ist, kann uns ja kaum befremden, wenn man daran denkt, daß z.B. der  $\alpha$ -Streifen des Oxyhämoglobins von *Planorbis* an 575  $m\mu$ , während

1) Der Streifen an 523  $m\mu$  ( $\beta$ -Streifen des Cytochroms  $c$ ) sieht hierbei im Vergleich mit demjenigen bei anderen Organismen bedeutend verschwommen aus. Ein analoger Fall wurde auch von FRIEDHEIM und BAER (1933) bei einem Bandwurm *Diphyllbothrium latum* und dessen Larve beobachtet, bei welchen zwar trotz dem Vorhandensein des  $\alpha$ -Bandes des Cytochroms  $c$  gar kein oder nur ein äußerst verwaschener Streifen an 530–520  $m\mu$  nachweisbar ist.

2) Dies erinnert uns an das von WARBURG und HAAS (1934) bei Bäckerhefe entdeckte Band an 583  $m\mu$ , das zwar erst bei  $O_2$ -Zugabe erscheint und ebenfalls durch CO verschoben wird. Dieser Streifen wurde auch von den genannten Autoren auf ein Oxygenierungsprodukt einer Ferro-Häminverbindung zurückgeführt, die aber nach der Lage des Bandes bei CO-Sättigung (600–590  $m\mu$ ) chemisch nicht ganz identisch mit dem gewöhnlichen Hämoglobin sein könnte.

derjenige von *Chironomus* an 578  $m\mu$  liegt.<sup>1)</sup> Besonders eigenartig für das Hämoglobin ist der zwischen den  $\alpha$ -Bändern von  $O_2$ - und CO-Verbindung liegende Abstand, für welchen wir bei Menschen- und *Paramecium*-Hämoglobin praktisch denselben Wert 6–7  $m\mu$  finden.

Für das Vorhandensein des Hämoglobins, und folglich des Protohäms, in *Paramecium*-zellen spricht ferner der Befund bei Zusatz des Pyridin-Hydrosulfits, bei welchem zwar folgende drei Streifen sichtbar werden:

- I. 593–582  $m\mu$ , Absorptionsmaximum: 588  $m\mu$ .
- II. 567–548  $m\mu$ , Absorptionsmaximum: 558  $m\mu$ .
- III. 533–518  $m\mu$ , Absorptionsmaximum: 526  $m\mu$ .

Der Streifen I stammt von Cytochrom  $\alpha$ , wie man es nach ROCHE und BÉNÉVENT (1936) schließen darf. Die Streifen II und III stellen bekanntlich den kombinierten Streifen aus Hämochromogen  $c$  und Protohämochromogen dar, welches letztere zweifelsohne aus dem Hämoglobin abgeleitet ist.

Setzt man den *Paramecium*-zellen Na-Hydrosulfit hinzu, so beobachtet man zuerst dasselbe Bild wie bei Evakuierung. Dauert die Einwirkung von Hydrosulfit etwas länger (einige Minuten), so tritt folgende merkwürdige Veränderung ein:

- (1) Der  $\alpha$ -Streifen (608  $m\mu$ ) verschwindet.
- (2) Die Absorption im Gelbgrün schmälert sich zu 553–549  $m\mu$ , mit einem Maximum ebenfalls an 551  $m\mu$ .
- (3) Im Gelb (583–571  $m\mu$ ) entsteht ein neues intensives Band mit einem Maximum an 577  $m\mu$ .
- (4) Die Absorption im Grün erweitert sich zu 543–519  $m\mu$ . Diese Absorption besteht wohl aus zwei Bändern mit dem Maximum an etwa 536 bzw. 523  $m\mu$ .

Setzt man nachträglich Chinon hinzu, so verschwinden zuerst die der  $c$ -Komponente zukommenden Absorptionen (551 und 523  $m\mu$ ), und es bleiben nur die Bänder an 577  $m\mu$  (583–571  $m\mu$ ) und 536  $m\mu$  (543–529  $m\mu$ ) zurück. Bei Zugabe einer größeren Menge des Chinons werden auch diese beiden Streifen unsichtbar, und anstatt deren tritt das Methämoglobinband an 633  $m\mu$  hervor. Alles deutet also darauf hin, daß die zuletzt erwähnten Streifen einem gewissen Derivat aus Hämoglobin zuzuschreiben sind. Solch eine Veränderung des Hämoglobins, ebenso wie diejenige der  $\alpha$ -Komponente, bei Reduktion mit Hydrosulfit ist bisher niemals berichtet worden, und bedarf einer künftigen Bearbeitung.

Nach alledem, was wir oben angeführt haben, sind wir zu dem Schluß berechtigt, daß in *Paramecium*-zellen drei Häminkörper, nämlich Cytochrom  $\alpha$ ,  $c$  und Hämoglobin, enthalten sind. Allerdings muß

<sup>1)</sup> Vergl. z.B. BARCROFT (1929); ANSON, BARCROFT, MIRSKY, u. OINUMA (1925).

aber, wie bei allen derartigen Betrachtungen der Fall ist, die Möglichkeit der Beimengung anderweitiger Hämverbindungen in spektroskopisch kaum nachweisbarer Menge in Erwägung gezogen werden. Bekanntlich ist es bei allermeisten tierischen sowie pflanzlichen Organismen der Fall, daß bei Anwesenheit des *a*- oder des *c*-Streifens des Cytochroms stets auch der mehr oder weniger deutliche *b*-Streifen beobachtet wird. Ausnahmefälle hierzu bat zuerst der von FRIEDHEIM und BAER (1933) untersuchte Bandwurm, der zwar nur den *c*-Streifen zeigt, und jetzt wiederum unser *Paramecium*, dessen Cytochromtyp (*a, c*) übrigens von allen bisher bekannten verschieden ist.

Andererseits stellt das *Paramecium* wohl die allerniedrigste Stufe der hämoglobinführenden Tiere dar, eine Tatsache, die biologisch sowie phylogenetisch ein besonderes Interesse beansprucht. Unser Befund, daß das Hämoglobin in *Paramecium*zellen auch bei Zugabe von Blausäure glatt reversible Oxygenierung und Desoxygenierung bewirkt, läßt uns annehmen, daß der Atmug von diesem Organismus ein gewisser CN-unempfindlicher Mechanismus inneohnt. Nähere Untersuchungen über die Atmung von *Paramecium* sind jetzt im Gange, worüber demnächst auch ein Bericht erstattet werden soll.

Es ist unsere besondere Freude, diese Arbeit unserem gemeinsamen Lehrer, Herrn Prof. honor. Dr. K. FUJII, zu seinem siebenzigjährigen Geburtstag ergebenst widmen zu dürfen. Herrn Prof. Dr. K. SHIBATA und Herrn Prof. Dr. H. HATTORI sprechen wir auch an dieser Stelle unseren wärmsten Dank aus für ihre stets liebenswürdige Unterstützung bei dieser Arbeit. Ebenso sei Herrn T. T. IIDA bestens gedankt, durch dessen gütige Hilfe die Beschaffung unseres Versuchsmaterials ermöglicht wurde.

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#### Literaturverzeichnis

1. Anson, M. L., Barcroft, J., Mirsky, A. E. u. Oinuma, S.: Proc. Roy. Soc. B. 97, 61, 1925.
2. Barcroft, J.: Die Atmungsfunktion des Blutes. 2. Teil. Berlin, 1929.
3. Doflein, Fr. u. Reichenow, E.: Lehrbuch der Protozoenkunde. 5. Aufl. Jena, 1927.
4. Friedheim, E. A. H. u. Baer, J. G.: Biochem. Zeitschr., 265, 329, 1933.
5. Fujita, A.: Biochem. Zeitschr., 273, 186, 1934.
6. Roche, J. u. Bénévent, M. T.: Bull. Soc. Chim. biol., 18, 825, 1650, 1936.
7. Tamiya, H. u. Yamaguchi, S.: Acta Phytochim., 7, 233, 1933.
8. Tamiya, H. u. Ogura, Y.: Acta Phytochim., 9, 123, 1937.
9. Yaoi, H. u. Tamiya, H.: Proc. Imp. Acad. Japan, 4, 436, 1928.
10. Warburg, O. u. Haas, E.: Naturwiss., 22, 207, 1934.

## Karyotype Analysis in the $F_1$ Hybrids of Cristate and Normal Forms of *Rumex acetosa*<sup>1)</sup>

By

Y. Sinotô

Tokyo Imperial University

A form of *Rumex acetosa*, newly called "cristate", first came into the writer's hands in 1934. The leaves of this plant present remarkable characteristics, being conspicuously wrinkled and folded (cf. Sinotô 1936, fig. 1). The plant was only female and crossed in the same year with a normal form having smooth leaves. By the first crossing twenty-five seeds were obtained all of which germinated in sand in Petri-dishes. Seven of the young plants were lost by accident, and the rest are being kept until now. All the  $F_1$  plants were found to have generally normal smooth characteristics in their leaves. The results of the crossing and of the karyotype analysis of the  $F_1$  progeny were preliminarily reported in 1935 and 1936 to the General Meetings of the Genetic Society of Japan (Sinotô, 1936, 1937).

### Materials and Methods

Root-tips taken from each individual of the  $F_1$  hybrids were fixed with either a modification of Navashin's fluid or Randolph's "Craf". The material was imbedded in paraffin, partly on the Randolph schedule using buthyl alcohol; sectioned to  $12\mu$  in thickness and stained with either Newton's iodine gentian violet or Heidenhain's iron-alum haematoxylin. Camera lucida drawings were made with a Zeiss achromatic objective, N.A. 1.3, and an Olympus  $15\times$  compensating ocular giving a magnification of ca.  $\times 5800$  reduced to one half in reproduction. Late prophase and metaphase figures were most suitable for the karyotype study.

### Karyotypes in the Parents

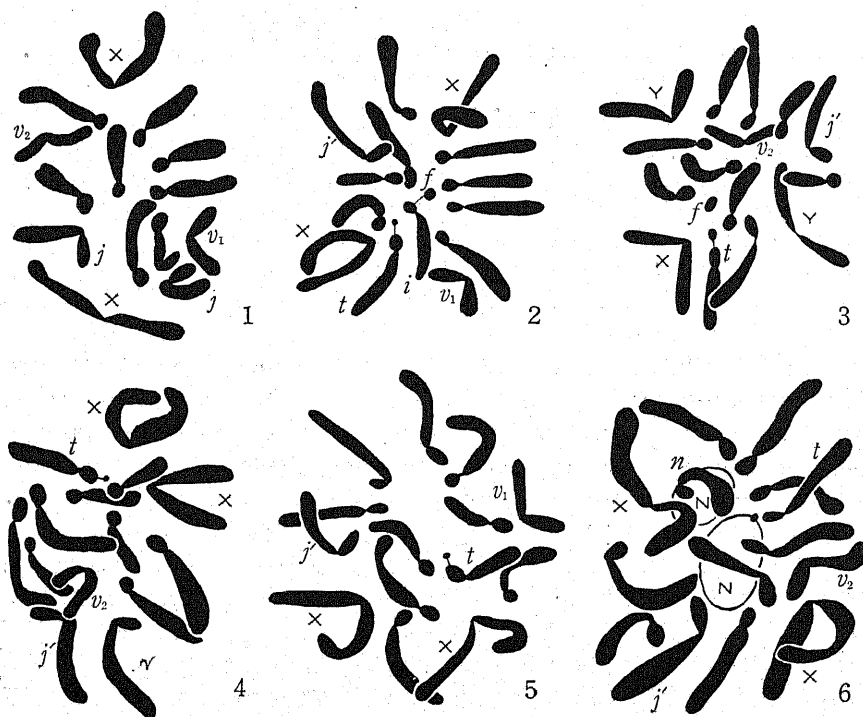
The cristate form (Cult. No.: S46-8-31-1-1) used as female parent has fourteen somatic chromosomes among which are three different type varieties, i, j and v, excepting a large V-shaped X. Two autosomes are typical of the type j, while the two v-chromosomes differ from each other in point of the fibre attachment, in the one ( $v_1$ ) this

1) Contributions from the Divisions of Plant-Morphology and of Genetics, Botanical Institute, Faculty of Science, Tokyo Imperial University, No. 189.

being median and in the other ( $v_2$ ) submedian. Autosomes belonging to the type i are not of the same size. Thus the karyotype is formulated as  $2n = 14 = 2x + 8i + 2j + v_1 + v_2$  (fig. 1). This karyotype corresponds to the type III of Kihara und Yamamoto (1931) or to the type DD of Ono (1935). The karyotype of the normal male parent (S46-8-30-1-1) seems to be the type VII of Kihara and Yamamoto (l.c.) or the type AA of Ono (l.c.) and has fifteen somatic chromosomes, of which twelve autosomes have two different type varieties, i and t, the latter bearing trabants.

### Karyotypes in the $F_1$ Plants (Table 1)

The karyotype expected to occur in the  $F_1$  progeny of the plant with a karyotype  $2n = 14 = 2x + 8i + 2j + 2v$  and the plant with a karyotype  $2n = 15 = x + 2Y + 10i + 2t$  was ordinarily a combination of these two parental karyotypes. Nevertheless the expected karyotypes, i.e.,  $\varphi = 2x + 9i + t + j + v$  and  $\delta = x + 2Y + 9i + t + j + v$  were only found in three (S46-8-32-1-3, 4 and 8; figs. 4, 5 and 14) out of the whole eighteen individuals.



Figs. 1-6. The somatic chromosome complements.  $\times$  ca. 2900. 1, a cristate form. 2-6, four hybrid plants. x, x-chromosome; y, y-chromosome; i, i-chromosome; j, j-chromosome;  $j'$ ,  $j'$ -chromosome; t, t-chromosome;  $v_1$ ,  $v_2$ ,  $v_1$ - and  $v_2$ -chromosomes; n, n-chromosome; f, fragment; N, nucleolus. Cf. Table 1.



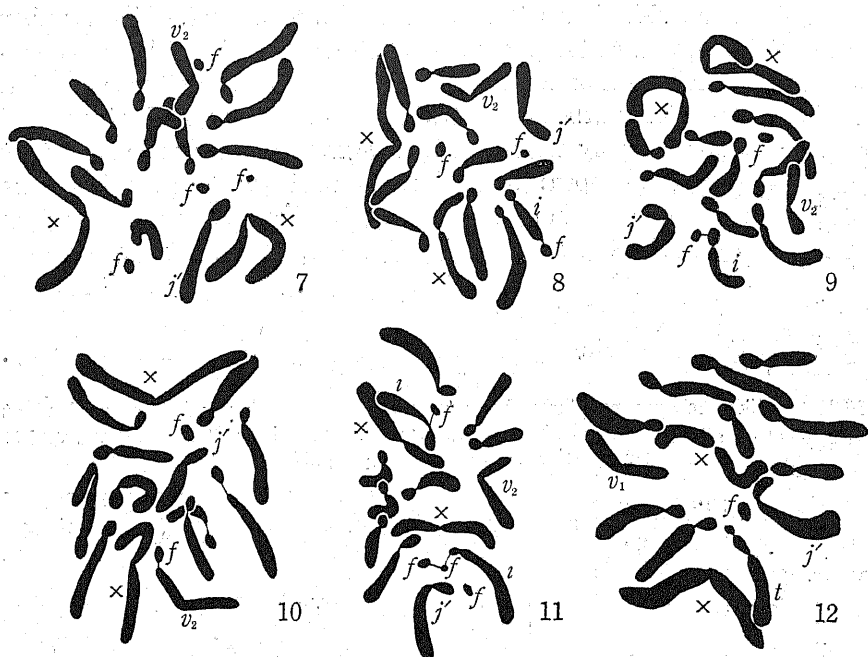
In six individuals no t-chromosomes were found. The number of nucleoli counted at telophases in all the  $F_1$  hybrids studied was always two which did not correspond to that of the t-chromosomes (cf. Table 1). Comparing the size of trabants in the  $F_1$  individuals we find that it is not generally uniform; in the extreme case it seemed that the trabants had disappeared or that only a seta was observed in some of the chromosomes, though in this latter case it was uncertain whether or not the trabant had disappeared. It was found that the trabant is attached to the nucleolus in the prophase (figs. 6, 13), and it was also observed that an apparently non-trabanted i-chromosome becomes attached at its end to the nucleolus (n-chromosome in fig. 6).

Table 1. Chromosome numbers, karyotypes, sex, and the number of nucleoli at telophase of  $F_1$  progeny of cristate *Rumex acetosa* ( $2n = 14 = 2x + 8i + 2j + v_1 + v_2$ ) ♀ × normal form ( $2n = 15 = x + 2y + 10i + 2t$ ) ♂

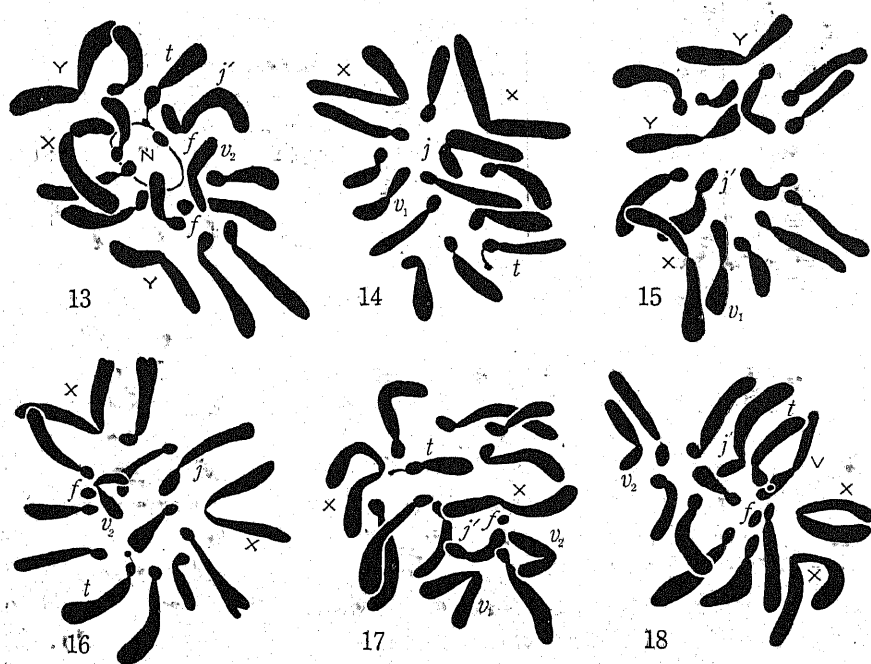
$F_1$ plants	chromosome number $2n$	sex	karyotype	number of nucleoli at telophase	figure
S46-8-32:1-1	14+f	♀	$2x + 9i + t + j' + v_1 + f$	2	2
" 2	15+f	♂	$x + 2y + 9i + t + j' + v_2 + f$	2	3
" 3	14	♀	$2x + 9i + t + j' + v_2$	2	4
" 4	14	♀	$2x + 9i + t + j' + v_1$	2	5, 6
" 5	14+2f	♀	$2x + 10i + j' + v_2 + 2f(1-5f)$	2	7-11
" 6	14+f	♀	$2x + 9i + t + j' + v_1 + f$	2	12
" 7	15+f	♂	$x + 2y + 9i + t + j' + v_2 + f$	2	13
" 8	14	♀	$2x + 9i + t + j + v_1$	2	14
" 9	15	♂	$x + 2y + 10i + j' + v_1$	2	15
" 10	14+f	♀	$2x + 9i + t + j + v_2 + f$	2	16
" 11	14+f	♀	$2x + 8i + t + j' + v_1 + v_2 + f$	2	17
" 12	14+f	♀	$2x + 8i + t + j' + v + v_2 + f$	2	18
" 13	14	♀	$2x + 10i + j + v_2$	2	19
" 14	15+f	♂	$x + 2y + 10i + j' + v_1 + f(1 \text{ or } 2f)$	2	20
" 15	15+f	♂	$x + 2y + 9i + t + j' + v_2 + f$	2	21
" 16	14+f	♀	$2x + 9i + t + j' + v + f$	2	22
" 17	14+f	♀	$2x + 9i + j + v_1 + v_2 + f$	2	23
" 18	14	♀	$2x + 10i + j' + v_1$	2	24

In two-thirds of the  $F_1$  plants, chromosome fragments(f) were observed (S46-8-32:1-1, 2, 5-7, 10-12, 14-17; figs. 2, 3, 7-13, 16-18, 20-23). Their size and number were various and almost constant in each individual, one fragment being most frequent. In one plant (S46-8-32:1-5), however, the number of the fragments showed a variation from one to five in different roots, i.e., one root had one fragment, an other from one to two and a third from two to five, two or three being counted most frequently (figs. 7-11). The origin of fragments is as yet unknown. They seem to have a strong tendency to become attached to the chromosomes. Some of them are free from the chromosomes (figs. 3, 7-12, etc.), while others are attached to the latter either at their terminals or lateral sides (figs. 2, 8-11). It was found that the fragments are attached to the chromosomes of various types, such as i, j, v and further x.





Figs. 7-12. The somatic chromosome complements of two hybrids.  $\times$  ca. 2900. Cf. Tabel 1.

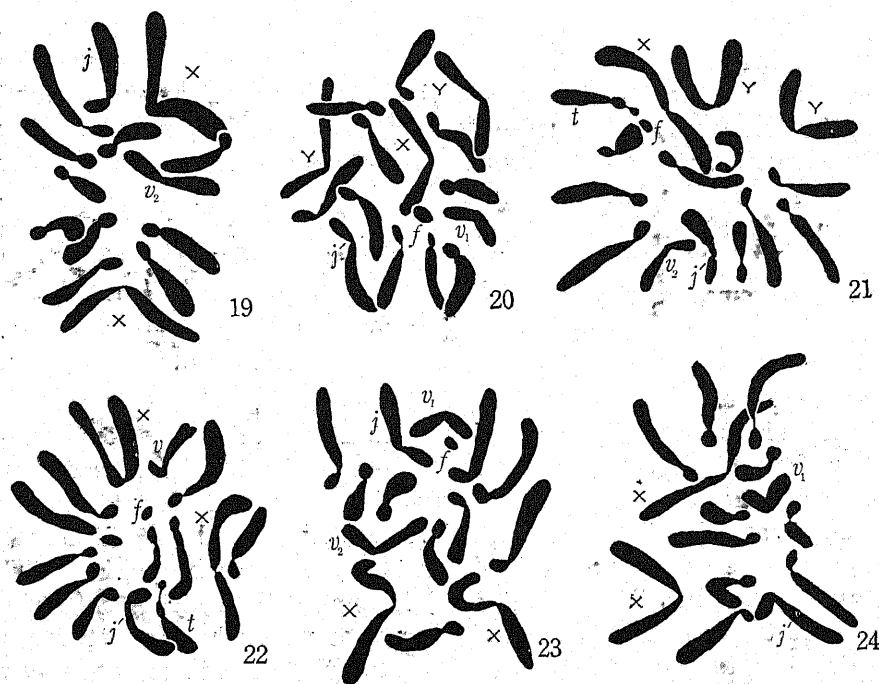


Figs. 13-18. The somatic chromosome complements of six hybrid plants.  $\times$  ca. 2900. Cf. Table 1. V (in fig. 18), V-chromosome of medium size.

The j-chromosomes in the hybrids must be direct descendants from the female parent and were expected to be similar in all respects to those of the parent. This, however, was not found to be the case. Only four hybrids (S46-8-32-1-8, 10, 13 and 17) were found to have the typical j-chromosomes similar to those of the mother (figs. 14, 16, 19, 23). In all the other hybrids the short arm of the j-chromosomes ( $j'$ ) is not so long as that of the typical j-chromosome or else it is only slightly distinguishable from the i-chromosomes.

The  $v_1$ - and  $v_2$ -chromosomes of the female parent probably synapse and disjoin normally in the meiosis of this plant as was often reported in other forms (types) with two v's of *Rumex acetosa* (cf. Yamamoto 1934, Satô and Sinotô 1935). In the present case of hybrids, therefore, an equal distribution of these v's was expected. Of eighteen  $F_1$  plants, we have found seven with  $v_1$  (S46-8-32-1-1, 4, 6, 8, 9, 14 and 18) and eight with  $v_2$  (S46-8-32-1-2, 3, 5, 7, 10, 12, 13 and 15), the ratio being almost up to expectation, though the total number was insufficient.

It is here noted that there appeared in the  $F_1$  hybrids, 1) two plants (S46-8-32-1-11 and 17) each with both  $v_1$  and  $v_2$  (figs. 17, 23), 2) one



Figs. 19-24. The somatic chromosome complements of six hybrid plants.  $\times$  ca. 2900. Cf. Table 1. - v (in fig. 22), an abnormal v-shaped chromosome.

plant (S46-8-32-1-12) including one  $v$  of medium size besides one  $v_2$  (fig. 18), and 3) one plant (S46-8-32-1-16) further which has neither typical  $v_1$  nor  $v_2$ , but has an anormally formed  $v$  which is scarcely distinguishable from  $i$ -chromosomes (fig. 22).

The sex and fertility of the hybrids have not yet been fully examined, and the result of only a preliminary survey of their sex is shown in Table 1.

### Considerations

It was expected that the hybrids between the cristate and normal forms of *Rumex acetosa* ordinarily have such a simple karyotype as is formulated as  $9i + t + j + v$ , the sex chromosomes being excepted. It was, however, found that only three out of eighteen hybrid plants came up to this expectation. The remaining hybrids presented markedly a nuclear polymorphism which is due to the following facts; namely 1) the appearance of the chromosome-fragments, 2) loss or transformation of the  $t$ -chromosomes, 3) the formation of new chromosome types, and 4) possible non-disjunction.

The fragments appeared newly in twelve plants out of all eighteen hybrid individuals and their number was from one to five, one being most frequently found. In one and the same individual we found that the number of fragments varied with different roots. One hybrid (S46-8-32-1-5) offers such an extreme case. There was also much variety in the size of the fragments. They were found either free from or attached to the chromosomes which may be either homologous or non-homologous  $x$ -,  $t$ -,  $i$ -, and  $v$ -chromosomes. Their positions of attachments were either terminal or side-ways. Considering the chromosome types in both parents and hybrids, it is deduced that the fragments had their origin in the  $t$ -,  $j$ -,  $v$ - and  $i$ -chromosome groups.

Each hybrid must normally contain one  $t$ -chromosome (trabant-chromosome), but six of the eighteen hybrids seemed to have no such chromosome. Why the  $t$ -chromosomes can not be detected in certain hybrids is at present uncertain, but there may be taken into consideration the fact that in this plant we often meet with the case of the trabants showing a size variation which often makes their detection difficult, and that the trabants might have disappeared (as in the case of amphiplasty in *Crepis*, Navashin 1927) or have been eliminated (as described by Satô 1936 in *Scilla*) in some of the hybrids, or (and) become transformed to the  $n$ -chromosomes which have no trabants but have a tendency to become attached at their end to the nucleolus. In the telophase of all eighteen hybrids there were always found two nucleoli which number does not correspond to that of the  $t$ -chro-

mosome. Though we do not intend here to go deep into the question of nucleolus-chromosome relation, the n-chromosome found in some of the present hybrids seems to have a similar situation to those non-trabanted chromosomes which have some connection with the nucleolus, such as observed in other plants by several authors, for example, in *Trillium Kamtschaticum* (Matsuura 1934), *Narcissus bulbocodium* var. *genuinus* (Fernandes 1936), and *Eremurus spectabilis* (Upcott 1936).

We can find the typical j-chromosomes similar to those of the mother plant in only four hybrid individuals, while in all other cases the newly appeared j'-chromosome can scarcely be distinguished from the i-chromosome.

Either of the two types of v-chromosomes of the mother parent is found actually unchanged in the hybrids, except in one hybrid (S46-8-32-1-16). The two v-types were distributed to the hybrids as expected generally, but in two hybrids (S46-8-32-1-11 and 17) both types were contained together. In these latter cases the phenomena are explained by non-disjunction which may have occurred in meiosis of the mother plant. However the formation of a v-shaped chromosome of medium size found in S46-8-32-1-12 or an anormal v-chromosome of S46-8-32-1-16 which closely resembles the i-chromosome can not be considered as being the results of non-disjunction. It may be more reasonable to assume that these new v-chromosomes have been produced by the process of structural reconstruction.

From the results of the observations set forth above it has become evident that in the first generation of the two different karyotypes of *Rumex acetosa* there have been brought forth considerable variations of karyotypes which were mostly due to the structural alteration of chromosomes, i.e., the appearance of the fragments, fragment-bearing chromosomes, j'-chromosome, large or anormal v-chromosomes as well as loss or transformation of t-chromosomes, though the course of production of such altered chromosomes has not yet been made clear.

*Rumex acetosa* has been known to have a considerable range of karyotypes with different constitutions for which the chromosome alterations have been considered to be mostly responsible by several authors. Besides these changes in chromosomes, repeated combinations of such different karyotypes may have been sources of still more new viable types, and the translocation and related alterations might have taken place in part through the effect of these repeated combinations as well as by introducing of a chromosome complement of one karyotype plant into a foreign cytosome, which may have disturbed the specific balance between the nucleus and cytosome.

### Conclusion

The leaf characteristic of the  $F_1$  hybrids between cristate and normal forms of *Rumex acetosa* was generally similar to that of the normal male parent. The karyotypes of the  $F_1$  hybrids showed a considerable variation instead of an expected simple combination of the parental karyotypes (cf. Table 1). The structural alteration of chromosomes is considered the main cause of these karyotype diversities found in the  $F_1$  plants produced through one time hybridization between different karyotypes of the species.

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Division of Genetics, Botanical Institute,  
Faculty of Science, Tokyo Imp. University

### Reference

- Fernandes, A. 1936. Les satellites chez les Narcisses II. Les satellites pendant la mitose. Bol. Soc. Bortoriana II (II sér.): 87-146.
- Jersen, H. W. 1936. Meiosis in *Rumex*. I. Polyploidy and the origin of new species. Cytologia 7: 1-22.
- 1936. Ditto. II. The origin and behavior of the so-called sex-chromosomes in *Rumex*. ib. 7: 23-34.
- Kihara, H. und Yamamoto, Y. 1931. Karyomorphologische Untersuchungen an *Rumex acetosa* L. und *Rumex montanus* Desf. Cytologia 3: 84-118.
- Matsuura, H. 1934. On the relation of chromosomes to nucleoli. Bot. and Zool. (Tokyo) 3: 1589-1594.
- Ono, T. 1935. Chromosomen und Sexualität von *Rumex acetosa*. Sci. Rep. Tôhoku Imp. Univ., 4 Ser., Biol., 10: 41-210.
- Satô, D. 1936. Chromosome studies in *Scilla*, III. SAT-chromosomes and the karyotype analysis in *Scilla* and other genera. Cytologia 7: 521-529.
- and Sinotô, Y. 1935. Chiasma studies in plants, III. Chromosome pairing and chiasma behaviour in the male *Rumex acetosa* with special reference to the tripartite sex-chromosome. Jap. Jour. Genet. 11: 219-226.
- Sinotô, Y. 1936. Cristate form of *Rumex acetosa* L. (a prel. note). Jap. Jour. Genet. 12: 24.
- 1937. Karyotype analysis in  $F_1$  progeny of cristate and normal forms of *Rumex acetosa* (a prel. note). ib. 13: 55-56.
- Takenaka, Y. 1931. Further reports of cytological and genetic investigations of *Rumex acetosa* L. I. New chromosome and chromosome-fragments. Bot. Mag. (Tokyo) 45: 475-489.
- 1936. Ditto. III. On male- and female-intersex plants. Jour. Chosen Nat. Hist. Soc. No. 21: 58-76.
- Yamamoto, Y. 1933. Karyotypes in *Rumex acetosa* L. and their geographical distribution. Jap. Jour. Genet. 8: 264-274.
- 1934. Karyogenetische Untersuchungen bei der Gattung *Rumex* I. Hetero- und Euploidie bei *Rumex acetosa* L. Cytologia 5: 317-336.
- 1935. Ditto. IV. Ein Fall von einfacher Translokation bei *Rumex acetosa* L. ib. 6: 407-412.
- 1936. Ditto. V. Nachkommenschaft der in bezug auf eine einfache Translokation heterozygotischer Pflanzen bei *Rumex acetosa* L. Jap. Jour. Bot. 8: 295-301.

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